PRODUCTION ECOLOGY OF *BRASSICA JUNCEA* (L.) CZERN. VAR. ENSABI

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PRODUCTION ECOLOGY OF *BRASSICA JUNCEA* (L.) CZERN.VAR. ENSABI

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DEDICATION

DEDICATE THIS DISSERTATION TO MY WIFE FIROOZEH FOR UNCONDITIONAL LOVE AND SUPPORT, PATIENCE AND ENCOURAGEMENT, TO MY CHILDREN FARAHNAZ AND FATEMEH AND TO THE MEMORY OF MY FATHER HABIB FALLAH TOOSI.

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ABBREVIATIONS

FEP	Fully exposed plant to Sunlight (mean midday radiation of 1834 μmole photon m^{-2} s^{-1})
PEP	Partially exposed plant to Sunlight (mean midday radiation of 367 μmole photon m^{-2} s^{-1})
DAP	Days after planting
PEG	Poly ethylene glycol
EtOH	Ethanol
NPK	Nitrogen, phosphate and potash
K_2O or K	Potash
P_2O_5 or P	Phosphate
N	Nitrogen
GA	Gibberellic acid
ABA	Abscic acid
T_b	Base temperature
T_o	Optimum temperature
T_c	Ceiling temperature

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PRODUCTION ECOLOGY OF *BRASSICA JUNCEA* (L.) Czern. VAR. ENSABI

ABSTRACT

The Malaysian brassica (*B. juncea* var. Ensabi) with its distinct pungent aroma and bitter taste is now cultivated as a local plant among the Malays and natives of Sabah and Sarawak. The species is also used as a popular kimchi by the locals.

Experiments were conducted to determine the effect of light, temperature regimes, salinity, drought stress and chemical media on seed germination and to assess clonal growth, structural demography and general growth patterns of *B. juncea* var. Ensabi. Fertilizer requirements, self-thinning, inter-plant spacing, assessment of the chemical nature and allelopathy activity of *B. juncea* var. Ensabi were also investigated in this study.

Seeds of *B. juncea* var. Ensabi were collected from Kuching, Sarawak. Most complete germination occurred at 25^{o}-30^{o}C and these were optimum for germination percentage and 30^{o}C was the best temperature for rate of germination for oven-dried seeds. No seed germination prevailed when exposed to chemical media. The hypocotyl indicated the optimum length growth was 22.8 mm at 25^{o}C on dry seeds under total darkness and for fresh seeds under same conditions was 23.7 mm at 25^{o}C. Light regime strongly influenced clonal growth of *B. juncea* var. Ensabi.

Yield and yield components of *B. juncea* var. Ensabi increased as the proportion of fertilizer increased from 0 to 150 kg of the total NPK plus 150 kg Nha^{-1} supplied more than farmers experience used. Based on these results N application higher than optimal rate had adverse effect on yield of *B. juncea* var. Ensabi.

Intra-specific competition had a profound effect on the number of individuals in a population, as illustrated by the Log of average leaf weight per plant plotted against

the log of density of survivors for a plant population. The leaf population's trajectory was held under a line of slope -1.29 ($R^2 = 0.63$, $p<0.05$).

The effect of inter-plant spacing on the growth of individual plants in an increased space and with equal sample size (number of plants) for each spacing gradient was investigated under varying plant spacing. The experimental design consisted of plants positioned in a polar coordinate grid with seven arcs, eighteen rays and an angle of 20^o. The distance between arcs increased exponentially from 6 cm to 43 cm. Rays were separated by an angle of 20^o. *B. juncea* var. Ensabi showed very high survival percentages even when planted at high densities for a period of 100 days after planting. Widely-spaced plants were significantly taller than the more closely spaced plants of the same cohort. Mean stem diameter, plant biomass, number of leaves and branches and pod/plant increased with plant (wider) spacing. These results support the concept of competition in the self-thinning rule.

Oil concentrations of the *B. juncea* var. Ensabi seeds was 34.6% and the protein concentration of the Ensabi seeds was 32.1%. The fatty acid profile of Ensabi showed on average oleic acid (C18:1) concentration in the oil produced from *B. juncea* var. Ensabi was 18.2% whereas the concentration of other fatty acids were: linoleic acid (16.9%), linolenic acid (5.5 %), eicosenoic acid (8.8%), erucic acid (42.0%), stearic acid (1.4%), and palmitic acid (2.5%), which was the major present as the major component.

In the study of allelopathy, aqueous extracts from fresh materials were assayed with concentrations of 0, 50, 100, 150, 200 and 300 g L^{-1}. The parallel concentrations of aqueous extracts of oven-dried materials used were 40, 80, 120, 160, 200 and 300 g/L. Inhibition of seed germination of radish prevailed at concentrations above 200 and 300 gL^{-1} of the aqueous extract of dried materials, while with ethanol extract similar inhibitions were observed at concentrations range of 14.28 – 30 gL^{-1}. Total inhibition of root and shoot growth was also observed in barnyard grass. Both species were

susceptible to plant extracts isolated from leaf, stem and root of *B. juncea* var. Ensabi. Root and shoot lengths of radish and barnyard grass were decreased with the extracts. Results are discussed from the viewpoint of possible use of the extracts as a natural herbicide to control barnyard grass and other susceptible weed species.

EKOLOGI PENGHASILAN/PENGELUARAN BAGI *BRASSICA JUNCEA* (L.) CZERN. VAR. ENSABI

ABSTRAK

Spesies *Brassica* di Malaysia yang mempunyai ciri-ciri seperti aroma yang menyerlah serta rasa pahit kini telah dibiakkan sebagai salah satu jenis sayuran tempatan di kalangan kaum Melayu dan masyarakat Bumiputera Sabah dan Sarawak. Spesies ini juga digunakan sebagai sejenis jeruk yang popular di kalangan masyarakat setempat.

Eksperimen yang telah dijalankan adalah bertujuan bagi mengenalpasti kesan-kesan cahaya, rejim suhu, kemasinan dan bahan-bahan kimia terhadap percambahan biji benih. Selain daripada itu, eksperimen yang dijalankan juga dapat menentukan pertumbuhan klonal, struktur demografi, kesan kemarau dan corak pertumbuhan am *B. juncea* var Ensabi. Keperluan-keperluan baja, penipisan semulajadi, ruang antara tumbuhan dan penilaian sifat kimia dan aktiviti allelopathi *B. juncea* var. Ensabi turut dikaji melalui eksperimen ini.

Biji benih *B. juncea* var. Ensabi ini telah dikumpul dari sekitar kampus Universiti Malaya, Kuala Lumpur, (3^o 8' N; 101^o 42' E), Malaysia . Kebanyakan percambahan lengkap berlaku pada suhu 25^o-30^oC dan julat suhu ini merupakan julat suhu terbaik bagi peratusan percambahan biji benih. Manakala suhu 30^oC pula, merupakan suhu optimum bagi percambahan biji benih yang telah didedahkan kepada pengasingan ketuhar secara oven-dried. Pendedahan biji benih kepada bahan-bahan kima menyebabkan tiada percambahan biji benih yang berlaku. Daripada pemerhatian yang dilakukan terhadap panjang hipotikol biji benih tersebut, didapati pertumbuhan optimum adalah pada 22.8 (mm) dan suhu 25^oC bagi biji benih kering dan dalam keadaan gelap manakala bagi biji benih segar di dalam suhu dan keadaan cahaya yang

sama pertumbuhan optimum adalah 23.7 (mm) at 25^{o}C. Didapati juga, faktor cahaya sangat mempengaruhi pertumbuhan klonal *B. juncea* var. Ensabi.

Di dalam eksperimen ini, hasil dan komponen hasil bagi *B. juncea* var. Ensabi telah ditingkatkan agar ia berkadar terus dengan jumlah baja yang telah ditingkatkan penggunaannya mengikut kadar 0 – 150 kg daripada jumlah NPK daripada jumlah baja yang biasanya digunakan oleh petani-petani yang berpengalaman. Berdasarkan kepada keputusan penggunaan N yang melebihi daripada dos optimal, didapati ia mempunyai kesan yang kurang baik/ merugikan terhadap jumlah penghasilan *B. juncea* var. Ensabi.

Persaingan intra-spesifik mempunyai kesan yang mendalam terhadap jumlah individu di dalam sesuatu populasi. Ini adalah seperti yang digambarkan di dalam graf log purata berat daun/ tumbuhan melawan log kepadatan tumbuhan yang terus hidup di dalam sesebuah populasi. Trajektori populasi daun tersebut diukur di bawah satu garisan slop -1.0 ($R^2 = 0.63$, $p<0.05$).

Selain itu, kesan jarak antara tumbuhan terhadap pertumbuhan tanaman individu di dalam keadaan pertambahan ruang dengan saiz sampel yang sama bagi setiap ruang kecerunan turut dikaji melalui variasi jarak di antara tanaman. Rekabentuk eksperimen tersebut terdiri daripada tumbuhan-tumbuhan yang ditanam dalam kedudukan grid polar tersusun dengan 7 lengkungan , 8 bayang-bayang yang bersudut 20^{o} . Jarak di antara lengkungan itu ditambah secara eksponen iaitu daripaa 6cm kepada 43cm. Bayang-bayang lengkungan itu pula adalah bersudut 20^{o}C. *B. juncea* var. Ensabi telah menunjukkan peratusan daya terus hidup yang tinggi walalupun di tanam di dalam keadaan yang berkepadatan tinggi dalam jangka masa 100 hari selepas penanaman. Pokok yang telah ditanamkan dalam jarak yang agak besar daripada pokok yang lain mempunyai ketinggian yang lebih tinggi berbanding dengan pokok-pokok yang telah ditanamkan secara berdekatan di antara satu sama lain di dalam kohort yang sama. Min diameter stem, biomass tumbuhan, jumlah daun dan ranting adalah berkadar terus

dengan pertambahan jarak penanaman di antara satu pokok dengan pokok yang lain didalam kohort yang sama. Ini membuktikan bahawa, wujudnya konsep persaingan dan penipisan semulajadi dalam bentuk penanaman ini.

Kepekatan minyak di dalam biji benih *B. juncea* var. Ensabi adalah sebanyak 34.6 % dan kepekatan proteinnya adalah sebanyak 32.1%. Profil lemak asid Ensabi menunjukkan minyak yang dihasilkan oleh B. juncea var. Ensabi mempunyai purata kepekatan oleic acid (C18:1) sebanyak 18.2% manakala kepekatan lemak-lemak asid yang lain adalah: linoleik asid (16.9%), linolenik asid (5.5 %), eikosenoik asid (8.8%), erusik asid (42.0%), estearik asid (1.4%), and palmitik asid (2.5%). Kesemua lemak-lemak asid ini merupakan komponen utama di dalam minyak yang di hasilkan Ensabi. Di dalam kajian allelopathi, ekstrak akues telah dicerakinkan daripada bahan mentah yang mempunyai kepekatan sebanyak, 50, 100, 150, 200 and 300 $gL^{-1.}$ Kepekatan sejajar bagi ekstrak akues biji benih oven-dried yang digunakan adalah 40, 80, 120, 160, 200 and 300 gL^{-1}. Penghalangan pecambahan biji benih lobak putih berlaku pada kepekatan melebihi 200 and 300 gL^{-1} di dalam ekstrak akues bahan-bahan kering. Manakala, bagi ekstrak etanol, diperhatikan penghalangan percambahan biji benih lobak putih berlaku pada julat kepekatan 14.28- 30 gL^{-1}. Penghalangan percambahan secara total turut berlaku di dalam perkembangan pertumbuhan barnyard grass. Kedua-dua spesies ini mudah dipengaruhi oleh ekstrak allelokimia yang telah diasingkan dari daun, batang dan juga akar. Akar dan panjang pucuk barnyard grass akan berkurangan dengan penambahan penggunaan ekstrak. Keputusan eksperimen telah dibincangkan daripada sudut pandangan yang menggunakan ekstrak sebagai racun semulajadi untuk mengawal pertumbuhan lobak putih dan barnyard grass melalui penahanan percambahan, pertumbuhan serta perkembangan biji benih tersebut.

CHAPTER 1

GENERAL INTRODUCTION

1.1 Prologue

The genus *Brassica* is one of 51 genera in the tribe *Brassiceae* and others closely related cruciferous crops are widely cultivated throughout the world as vegetable crops for human consumption, condiments and spices for improved flavour of human diets, and as fodder crops for livestocks. However, the largest cultivation of these crops is for edible vegetable oil production (Gomez-Campo 1980; Downey and Rimmer 1993; Muñoz-Valenzuela *et al*. 2002; Pua and Douglas 2004). Many crop species are included in the Brassica genus, which provide edible roots, leaves, stems, buds, flowers and seeds.

Brassica juncea is a member of the subtribe *Brassicinae* of the tribe *Brassiceae* of the Cruciferous (*Brassicaceae*) family sometimes referred to as the mustard family. The name "cruciferous" comes from the shape of its flowers, which have four diagonally opposite petals in the form of a cross. *B. juncea* is a popular leaf vegetable in the Far East (Palada and Crossman 1999). Although mustard exhibits several desirable agronomic characteristics with high oil content and quality that can be used for oil production and green manure specifically for biofumigation. It is also used as a condiment (Gunasekera 2001; Gunasekera 2006).

Brassica juncea var. Ensabi, is a local mustard plant. This crop is popular among varieties in Malaysia and slightly smaller than the normal mustard plant with aromatic and bitter taste. The ecology and agronomy of Ensabi are less well-known and the purpose of this thesis is to gather and provide new insights necessary to develop the agronomy and production ecology of *B. juncea* var. Ensabi.

The present workwas carried out with the below objectives:

1. To study the environmental effect (*e.g.* temperature, light, drought stress, salinity, etc) on seed germination and seedling growth of Ensabi.

2. To determine the effect of N, P, K on some morphological characters, yield and yield components of Ensabi.
3. To identify the ecological affects of plant population on general growth pattern and intra-competition to understand the mechanisms of competition at the individual plant level of Ensabi.
4. To study agronomic traits and allelopathic effect of Ensabi.
5. To assess the chemical nature of Ensabi.

1.2 Vegetable industries in the world

Vegetables are produced in almost countries all over the world. Global vegetable fresh production amounted to a total of 240 million tones. This is according to statistics from the Food and Agriculture Organization of the United Nations (FAO, 2008). Asia cultivates by far the most vegetables in the world and has also shown strongest growth over the last decade. Much of this growth can be attributed to China which cultivate over 22 million hectares of vegetable crops out of a global total of 52 million hectares (**Fig 1.1**)

The vegetable brassicas have been cultivated in Europe since ancient time from where they have spread to other parts of the world (Monteiro 1999). *Brassicas* include many different morphotypes, to well adapted to temperate climates and requires quite simple cultivation techniques to produce abundant and nutritious food for man and domestic animals. In the cold-winter regions of Europe white cabbage is processed as sauerkraut, while in the mild-winter regions, brassicas are the most important vegetables during the cool season. In Korea, Chinese cabbage is used to prepare "kimchi", a very popular national preserve (Monteiro 1999). World trade of fresh vegetables **(Fig 1.2**) has been increasing in the last few years in 2005, world exports of fresh vegetables reached US $1.6 billion.

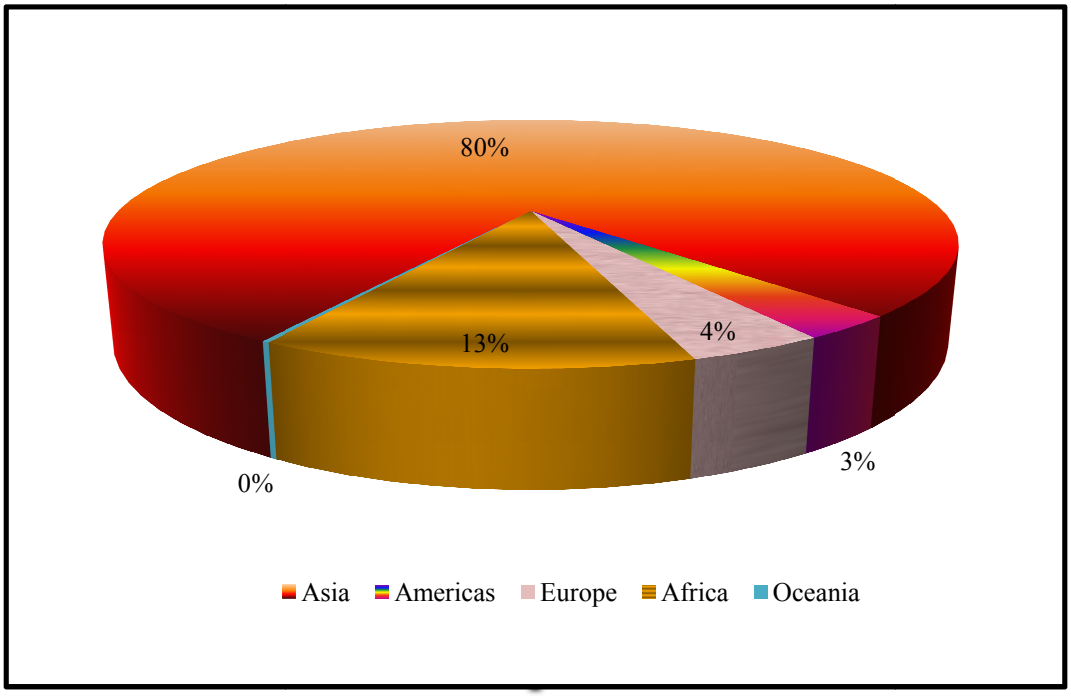

Fig. 1.1. World fresh vegetables production in the different continents (FAO Statistics 2008).

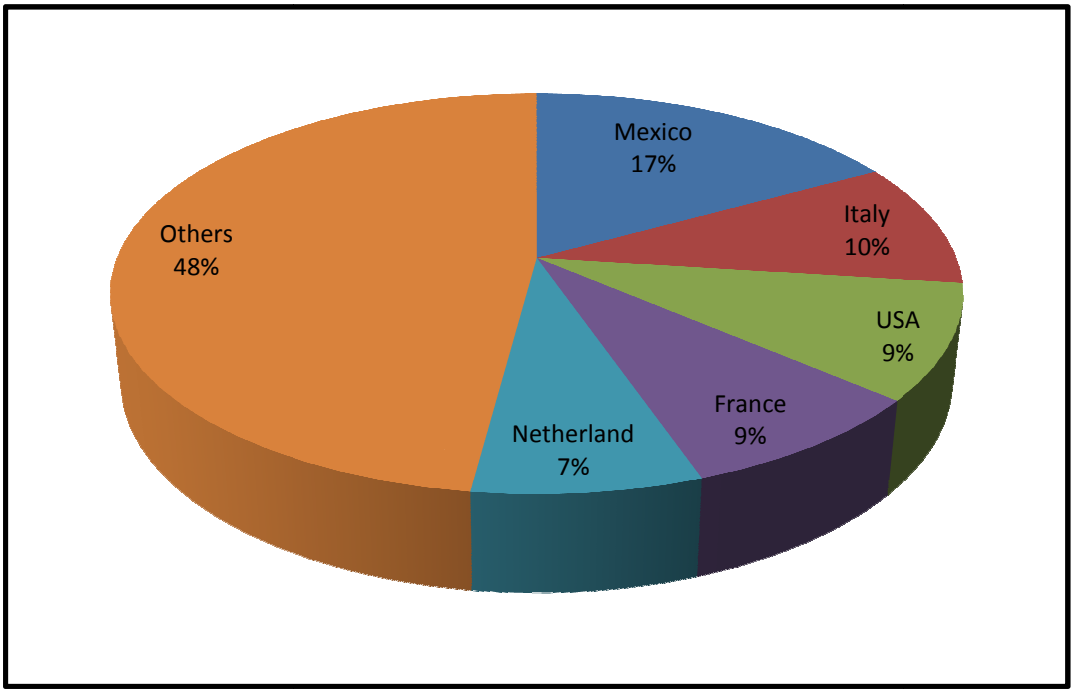

Fig. 1.2. Exports of fresh vegetables by value in the world in 2005 (FAO Statistics 2007).

In Asia, *B. campestris* is the most cultivated species because of the great importance of Chinese cabbage. Turnip and turnip greens, which also belong to *B. campestris*, are cultivated world-wide but have much less economic importance (Monteiro 1999).

1.2.1 Vegetables in *Brassica* family

Vegetable brassicas are an important and highly diversified group of crops grown world-wide belonging mainly to the species *Brassica oleracea* and *B. campestris* (Monteiro 1999). Where world trade tends to dominate the vegetable market, local availability is no longer a comparative advantage for brassicas. However, vegetable brassicas have new appeal in developed countries due to the potential for market diversification of some new crop types, and to the use of cauliflower and broccoli as salad crops. With an increasing number of consumers aware of the importance of diet on human health, the potential benefits of a diet with green vegetables have given brassica vegetables a better image during recent years. In less developed countries brassicas are still considered as an important source of cheap and abundant food (Monteiro 1999).

The mustard family contains a number of important vegetables—broccoli, brussel sprouts, cabbage, cauliflower, collards, kale, and kohlrabi—all members of *Brassica oleraceae* and comprising a group of vegetables called the cole crops, a term that probably reflects the fact that they are principally stem plants (Britannica 2008).

Mustard greens are jampacked with nutrients and provide good to excellent amounts of 9 vitamins, 7 minerals, dietary fiber and protein have been grown and consumed for more than 5,000 years. Mustard greens are a notable vegetable from China to Southern American. While India, Nepal, China and Japan are among the leading producers of mustard greens, a significant amount of mustard greens are grown in the United States as well. Mustard greens are an excellent source of many vitamins including vitamin A,

vitamin C, folate, and vitamin E. They are also an excellent source of the mineral manganese. In addition, mustard greens are an excellent source of dietary fiber (Mateljan 2006).

In Malaysia, several types of fresh vegetables are produced and raw consumed, locally known as 'ulam' or equivalent to 'salad' in other countries. Usually these vegetables are mixed with other ingredients such as chillies, grated coconut and rice. This food is popular among the Malay ethnic group (Noorzaleha 2003), Malaysians also consume vegetables such as spinach, kale, and swamp cabbage in cooked forms. Cooking may affect the antioxidant content in vegetables, especially components such as tocopherol, carotenoids, ascorbic acid and polyphenols (Amin 2004).

1.3 Oilseed industry in the world

Oil crops have been cultivated since antiquity and are now grown all over the world and in many economies they are a vital part of the agriculture sector. They are very important component of semi-tropical and tropical agriculture, providing easily available and highly nutritious human and animal food (Weiss 1999). Oilseeds and edible oils are two of the most sensitive essential commodities.

Four major *Brassica* oilseed species widely cultivated throughout the world are, *Brassica napus* L. (rape), *B. rapa* L. (turnip rape), *B. juncea* (L.) Czern. and Coss., and *B. carinata* Braun (Downey and Rimmer 1993) while other cruciferous spices such as *Sinapis alba* (white mustard) have been grown for oil production in few countries.

Table 1.1. Production of mustard by harvested area in certain countries. Source: (FAO STATISTICS 2007).

Country	Harvested Area (hectares)	Production (tonnes)
Canada	170000	112000
Nepal	190000	141000
Myanmar	90000	60000
Ukrain	42000	21000
China	22000	16500
World	675970	417337

1.4 Taxonomy, nomenclature and spices of *Brassicacea* family

The *Brassicaceae* (*Cruciferae family*), which includes about 3500 species and 375 genera, is a large and diverse group of plant species that are economically very important throughout the world. It is one of the 10 most economically important plant families that have been extensively altered and domesticated by humans. The *Brassicaceae* crops are used as sources of oil, vegetables, mustard condiments, and fodder (Jessop and Toelken 1986; Evans and Sorger 1966; cited by Warwick *et al*. 2008). The name crucifer comes from the shape of flowers, with four diagonally opposite petals in the form of a cross.

The major brassica species which cultivated around the world as a source of vegetable oil are: *Brassica napus* L., *Brassica rapa* L., *B. juncea* L., and *Brassica carinata* (Downey and and Rimmer 1993). The botanical relationships between the *Brassica* species were established by means of taxonomic studies carried out in the 1930s (Kimber and McGregor 1997). *Brassica napus* L. (*n*=19, AACC), *B. juncea* L. (*n*=18, AABB) and

Brassica carinata L. (*n*=17, BBCC) are three species with higher chromosome numbers, that amphidiploids derived from the diploid species *Brassica nigra* (L.) Koch *(n*= 8, BB), *Brassica rapa* L. *(n*=10*,* AA) and *Brassica oleracea* L. (n=9, CC). These six of the most important *Brassica* species are closely interrelated. The relationship between these species can be represented as a triangle with the three diploid species forming the points of the triangle, and the amphidiploid species (crosses between the first three) on the sides (**Fig. 1.3**).

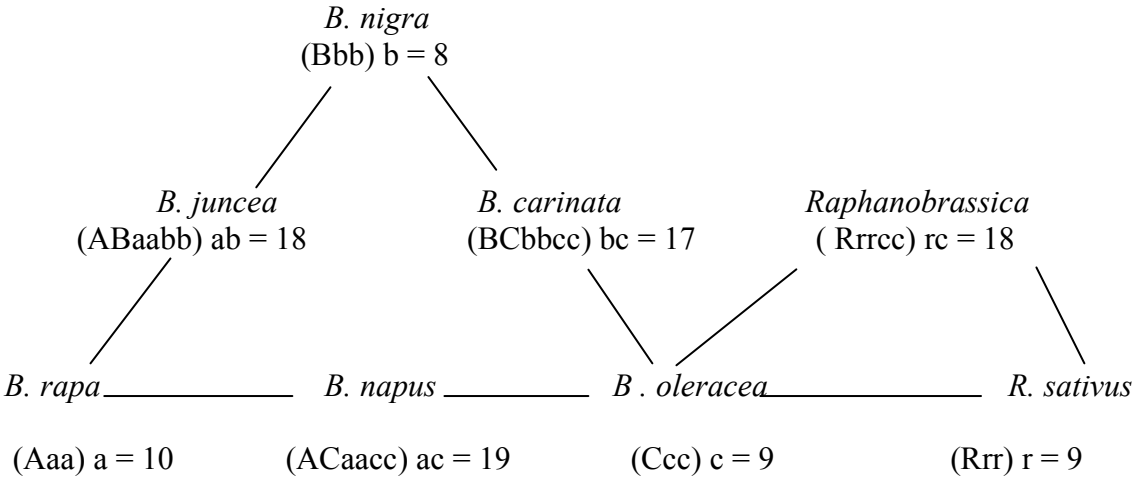

Fig. 1.3. Interrelationships between several *Brassica* species, and the genus *Raphanus* (World Food Program)

Brassica vegetables are of great economic importance throughout the world and different species are utilized. The principal *Brassica* vegetable species is *B. oleracea*, which provides a large range of unique cole and cabbage types that include headed cabbages, brussel sprouts, cauliflower, broccoli, and others.

1.4.1 Taxonomy and origin of *B. juncea*

Mustard was one of the first crops domesticated by man and may be among the oldest cultivated plants known to man (Sovero 1993; Johnston *et al*. 2002). The name mustard is given to several cruciferous plants of the genus *Brassica* that includes broccoli,

cabbage, cauliflower, turnips and radishes, the most common being white mustard (*B. hirta Moench* or *Sinapis alba L.*), black mustard (*B. nigra* Koch) and Indian or leaf mustard (*B. juncea* Coss.).

There are also many herbs of the same family called mustard, and have more or less flavor of the true mustard; as, bowyer's mustard (*Lepidium ruderale*); hedge mustard (*Sisymbrium officinale*) and; mithridate mustard (*Thlaspi arvense*); tower mustard (*Arabis perfoliata*); treacle mustard (*Erysimum cheiranthoides*).

Brassica juncea L. Czern. and Coss., (n = 18) is an amphidiploid species derived from interspecific crosses between *B. nigra* (n = 9) and *B. rapa* (n = 10) is generally thought to have originated in the Middle East where wild forms of *B. juncea* have been found in the Near East and in southern Iran and *B. rapa* and *B. nigra* overlap in the wild (Kimber and McGregor 1997).

It is grown as an oilseed in India (Indian mustard), and as a leaf vegetable in China where leaf mustards have their greatest differentiation. Also, root type (turnip-like) forms var. *napiformis* are cultivated in China. However, China cannot be considered as a centre of origin for *B. juncea* because the two parent species, *B. nigra* and *B. rapa*, were never found as wild species in that country. The Chinese *B. juncea* forms are yellow-seeded, in contrast to the brown-seeded Indian types which also have a larger seed size. The yellow-seeded *B. juncea* types are grown as an oilseed in the Ukraine. Indian oilseed types contain primarily 3-butenyl glucosinolate in their seeds and vegetative tissue, while *B. juncea* from China contains only 2-propenyl (allyl) glucosinolate, and only trace amounts of 3-butenyl glucosinolate. *B. juncea* mustard is also grown for the production of condiment mustard in western countries with major production in western Canada (brown and oriental mustard).

Brassica juncea is distinguished by the colour of its seed. The brown-seeded cultivars are known as brown mustard, while the yellow-seeded cultivars are referred to as yellow or Oriental mustard. It is well adapted to drier conditions and relatively matures early (Kimber and McGregor 1997).

Brassica juncea can be up to one meter or more in height, with long, erect or patent branches, lower leaves petioled, green, sometimes with a whitish bloom, ovate to obovate, variously lobed with toothed, scalloped or frilled edges, lyrate-pinnatisect, with 1–2 lobes or leaflets on each side and a larger sparsely setose, terminal lobe; upper leaves subentire, short petioled, 30–60 mm long, 2–3.5 mm wide, constricted at intervals, sessile, attenuate into a tapering, seedless, short beak 5–10 mm long, usually grown as an annual or biennial (Duke 1983).

1.4.2 *Brassica juncea* as a vegetable crop

Brassica juncea, that includes the vegetables mustards, has some economic relevance in Far Eastern countries but is a minor crop at the world level (Monteiro 1999). Leaf mustards (*B. juncea*) are consumed in high quantities in China and other Asian countries. There is a range of different leaf types grown var. *japonica*, var. *integrifolia*, and others. The greatest differentiation in plant types is found in the Sichuan province of China. The leaf mustards are local vegetable crops and there is very little trade (Pua and Douglas 2004). Young tender leaves of mustard greens are used in salads or mixed with other salad greens. Older leaves with stems may be eaten fresh, canned or frozen, for potherbs, and to a limited extent in salads. Mustard greens are often cooked with ham or salt pork, and may be used in soups and stews. Although widely and extensively grown as a vegetable, it is being grown more for its seeds which yield an essential oil and condiment (Duke 1983).

Mustard [*B. juncea* (L.)] is an annual vegetable, widely grown for its seed. However, its glabrous young leaves are extensively eaten raw or cooked like spinach or turnip. Mustard has been reported as a natural traditional treatment to reduce headache, inflammations and hemorrhages, and their ingestion may impart a body odor to repel mosquitoes (Duke 1983).

1.4.3 *Brassica juncea* as an oilseed crop

The oilseed *Brassica* spices (*B. rapa, B. napus or B. compestrise and B. juncea*) are now the third important source of edible vegetable oils after the palm and soybean oil (Zhang and Zhou 2006).

Cultivars of *B. juncea* are grown as green vegetables and for the production of oilseed (Downey and and Rimmer 1993). To compare the oil, the fatty acid profile of zero erucic lines of *B. juncea* was more unsaturated than the canola cultivars of *B. napus or B. rapa*. The oleic acid content of the zero erucic lines of *B. juncea* was about 15% less and the linoleic acid about 15% higher than that of *B. napus*. This was considered undesirable because of the poor stability of unsaturated oil and would have prevented the use of *B. juncea* oil as canola. Efforts were made to change the fatty acid profile through interspecific crossing (Agnihotri *et al*. 1995; Raney *et al*. 1995; Kaushik and Agnihotri 2000), mutagenesis and genetic engineering.

Indian mustard [*B. juncea* (L.) Czern.] is the major oilseed on that subcontinent and is a more productive oilseed than canola (*B. napus* L.) in hot regions of Russia, India, China, and Canada with somewhat unreliable rainfalls, whereas canola is the higher yielding species in more temperate, more wet regions (Oram *et al*. 2005).

1.5 Distribution of *B. juncea*

The species a true indigene Western Europe and with highest diversity in Irano-Turanian, Mediterranean and temperate regions, center and east of Asia. Cultivated species, which are grown worldwide, many of the wild species grow as weeds, especially in North America, South America, and Australia. The Flora of China has shown that brassica is distributed on all continents except Antarctica. In the Mediterranean area 113 genera occur of which 21 (17%) are endemic, and 625 species of which 284 (45%) are endemic. The Irano-Turanian region has 147 genera of which 62 (42%) are endemic and 874 species of which 524 (60%) are endemic, while in the Saharan-Sindian region there are 65 genera, 19 (30%) being endemic and 180 species, 62 (34%) of which are endemic (McMurray 1999). Most members share a suite of glucosinolate compounds that has a typical pungent odor usually associated with cole crops. Whilst some members have seeds with a high erucic acid content, making them unsafe to eat in large doses, all members of this family that often in sulfur compounds and in vitamin C are edible (Gomez-Campo 1980).

Southeast Asia is one of the eight centers of the origin of crops and *B. juncea* is native to the region (Engle 2003). The Malaysian brassicas species include *Brassica oleracea, B. chinsis, B. juslenius, B. rapa, B. peinensis* and *B. juncea* also known as mustard green, leaf mustard, Indian mustard, brown mustard and Chinese mustard with the equivalent Malay names of *Sawi, Sawi pahit* and Ensabi (Barlo 1999).

Table 1.2. Classification of *Brassica juncea* taxonomy (USDA 2008)

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Dilleniidae*
Order	*Capparales*
Family	*Brassicaceae* – Mustard family
Genus	*Brassica* L. – mustard
Species	*Brassica juncea* (L.) Czern. – India mustard

The primary center of its origin is thought to be central Asia (northwest India), with secondary centers in central and western China, eastern India, Burma, and through Iran to Near East. It has been cultivated for centuries in many parts of Eurasia. The principle growing countries are Bangladesh, Central Africa, China, India, Japan, Nepal, and Pakistan, as well as southern Russia north of the Caspian Sea. In addition to the cultivated species, which are grown worldwide, many of the wild species grow as weeds, especially in North America (United States and as a principle weed in Canada), South America

(common weed in Argentina), Mexico, Fiji and Australia. Mustard is widely distributed as a cultivar and escape in subtropical and temperate climates (Downey and and Rimmer 1993).

Ranging from Boreal Wet to Tropical Thorn through Tropical Wet Forest Life Zones, Indian Mustard is reported to tolerate annual precipitation of 500 to 4,200 mm, annual temperature of 6 to 27°C, and pH of 4.3 to 8.3. Mustard green is a hardy, cool-season vegetable, growing well at monthly average temperatures of 15 to 18°C. Rai is grown mostly as a rainfed crop but grows well in some dry parts of northern and central Africa, northern India, and the interior of China. It is moderately tolerant of soil acidity, preferring a pH from 5.5 to 6.8. Thrives in areas with hot days and cool nights and is fairly drought resistant (Duke 1983). This plant typically grows in full sunlight under mesic to dry conditions. It is not fussy about the characteristics of the soil, and can often be found in clay-loam or gravelly sites. However, fertile soil produce larger plants. Disease rarely bothers this plant in the wild, although various insects often chomp holes in the foliage (Hilty 2007).

1.6 Ecology and description of *B. juncea*

Brassica juncea is a summer or winter annual about 1-4' tall, branching occasionally in the upper half (Illinois Wildflower 2008). Initially, there is a rosette of basal leaves, but during warm weather this plant has a tendency to bolt and develop round and hairless flowering stems. The alternate leaves are up to 12" long and 4" across. The typical leaf is pinnatifid, tapering gradually to a stout petiole and becoming broader toward the large terminal lobe. There is a stout central vein along its length. A few of the upper leaves may be unlobed. These leaves are bluish green (usually), glabrous, and glaucous, while their margins are undulate or dentate. The upper stems terminate in narrow racemes of yellow

flowers. Each flower is about ½" across, consisting of 4 yellow petals, 4 yellowish green sepals, a short green pistil with a knobby stigma, and several stamens with yellow anthers".

"The rounded petals are slightly notched at their tips, and have faint veins running across their length. The pedicel of each flower is about 1/3" long or longer. The blooming period usually occurs from late spring to mid-summer, but some plants bloom during the late summer or early fall. Individual plants remain in bloom for about a month. Each flower is replaced by a hairless silique (*i.e.*, a seedpod) that is cylindrical and held more or less erect. A mature silique is about ¾–1¼" long, and has a conspicuous beak at its tip. There are 2 faint lines running along its length. The small round seeds are arranged in a single row within each silique. The root system consists of a taproot. This plant spreads by reseeding itself and the Flora of China described *B. juncea* as: Herbs annual, (20-) 30-100 (-180) cm tall, pubescent or rarely glabrous, glaucous or not, sometimes with fleshy taproots. Stems erect, branched above. Basal and lowermost cauline leaves long petiolate; petiole (1-) 2-8 (-15) cm; leaf blade ovate, oblong, or lanceolate in outline, (4) 6-30 (80) × 1.5-15 (28) cm, lyrate-pinnatifid or pinnatisect; terminal lobe ovate, repand, dentate, or incised; lateral lobes 1-3 on each side of midvein, much smaller than terminal lobe, crisped incised, dentate, repand, or entire. Seeds dark to light brown or gray, globose, 1-1.7 mm in diam, minutely retic".

Fig. 1.4. Schematic representation of the life cycle of *Brassica juncea* var. Ensabi: (a) mature plant, (b) flower, (c) pod, (d) root, (e) seed.

The *B. juncea* seeds are dark to light brown and reddish with small in size, less than 2 mm in diameter, oval shape and predominate netting; texture is similar to a golf ball in surface (Duke 1983; USDA 2008).

1.7 Agronomy and general growth patterns

1.7.1 Seed germination and seedling growth

The ecophysiological study of seeds allows the understanding of mechanisms regulate seed longevity in the soil, dormancy breaking, germination and plant establishment in natural conditions. This is an important aspect of plant biology, when studying species which only become established in disturbed sites of mature vegetation (Vazquez-Yanes and Orozco-Segovia 1984; Leite and Takaki 2001).

Germination and seed vigour are two important characteristics of a seed. They greatly affect the rate of stand establishment, density of stand, rate of seedling and plant growth, the time of flowering, maturity, uniformity of the crop and yield. Seed vigour refers to the ability of seed to germinate, emerge, grow rapidly and produce a normal seedling to establish a good crop under a wide range of environmental conditions (Basra 1995; Hrstkova *et al*. 2006), Vigour is not a single property of a seed but the sum total of the properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling establishment (McDonnell *et al*. 1982; Dent *et al*. 2004). Non-dormant, vigorous seed will be expected to germinate rapidly. However, when seedbed conditions do not permit germination immediately after sowing, vigorous seeds are capable of surviving until conditions improve and then produce vigorous, healthy seedlings and a good crop (Ellis 1992; Schmidt 2000).

Planting crops early in the spring in an attempt to produce early maturity is often desirable. But, the risk of poor stands from poor field germination associated with high soil moisture content, low soil temperature and microbial activity is increased by early planting. However, high-vigour seeds should provide rapid, uniform and higher field emergence than low-vigour seeds under a wide range of field conditions (Basra 1995; Copeland and McDonald 2001), and facilitate early seeding. For modern crop production and efforts of conservation high seed quality as essential is widely accepted (Hampton and Hill 2002; Dent *et al*. 2004).

Various definitions of seed germination have been reported. According to one of them seed germination is a complex physiological process that commence with the uptake of water by the quiescent dry seed and terminate with the elongation of the embryonic axis (Bewley 1997), germination is defined from the physiological perspective as "the emergence of the radicle through the seed coat"(Copeland and McDonald 2001), germination events and subsequent establishment are controlled by nuclear and maternal genetics, and current and maternal environments (Meyer and Ppendleton 2000; Baskin 2005).

However, to the seed analyst, define germination as "the emergence and development from the seed embryo of those essential structures which, for the kind of seed in question, are indicative of the ability to produce a normal plant under favourable conditions".

The visible sign that germination is complete is usually the penetration of the structures surrounding the embryo by the radicle. The result is often called visible germination (Bewley 1997). Radicle emergence is considered as the completion of germination. In order to germinate, seeds must be viable and viable seeds can germinate

under favourable conditions, but viable seeds may not germinate if it is prevented by some form (or forms) of seed dormancy. If will germinate any dormancy which may be present are removed (Hampton and Hill 2002). Seed "viability" is defined as "the property of the seed that enables it to germinate under conditions favourable for germination provided that any dormancy in the seed is removed before the germination test" (Basu 1995).

Emergence in the field is one of the most critical factors in obtaining maximum yield in most crops. The rapid establishment of optimum plant stands under widely differing environmental conditions in the field is required to maximise yield (Dornbos *et al*. 1989; Reddya *et al*. 2003).

The standard germination test, in which the seed is exposed to an ideal germinating temperature and optimum conditions, is the most commonly used measure of commercial seed quality (Schell *et al*. 1991). The result of standard germination test usually is a reliable predictor of seedling emergence potential and is correlated well with field emergence when field conditions at planting are near optimum (Copeland and McDonald 2001). However, the value of the standard germination test is frequently questioned since optimal temperatures and conditions are seldom encountered in the field (Schell *et al*. 1991). Standard germination results usually overestimate field emergence under suboptimal field conditions (Copeland and McDonald 2001).

Germination can be considered as resumption of active growth by the embryo resulting in the rupture of the seed coat and the emergence of young plant. This definition presumes that after seed formation and development, the seed has been in a resting period in which it is in a relatively inactive state with a low metabolic rate. The seed can remain in this resting state until environmental conditions trigger the resumption of growth of the embryo (Copeland and McDonald 2001). During germination, most seeds undergo a specific

sequence of events of imbibition, enzyme activation, initiation of embryo growth, rupture of the seed coat, and emergence of seedling (Copeland and McDonald 2001). When dry viable seeds imbibe water, metabolism quickly recommences and a chain of events such as respiration, enzyme and organelle activity, and RNA and protein synthesis are initiated (Bewley and Black 1994).

Both agronomic and seed quality characteristics are important in oilseed crops. Agronomic characteristics such as high seed yield, early maturity, and lodging resistance improve the efficiency of production. But seed quality characteristics such as high oil and protein concentration and chemical composition of oil improve the value of the product. Oil and protein concentration, and the quality of the seed oil are directly related to marketability. Seed yield and oil concentration improvement are the primary objectives in breeding oil crops, including *B. juncea* (Rathke *et al*. 2006).

Environmental factors regulating germination include temperature, water, and oxygen for nondormant seeds, along with light and a suitable chemical environment for dormant seeds (Bewley and Black 1994; Baskin 2005). A consideration of the ecology of a species may sometimes help to provide some guidance as to the appropriate germination test conditions. At the simplest level, tropical species generally require higher temperatures for germination than temperate species. However, there are other more subtle responses which relate to the strategy of the plant in relation to its natural environment and a consideration of these may help in the development of germination test conditions which minimize dormancy (Ellis 1992).

1.7.2 Effect of light and temperature on seed germination

Temperature is one of the environmental factors that regulate germination of nondormat seeds, along with light for dormant seeds (Bewley and Black 1994; Leon and

Owen 2003; Baskin 2005). The light requirement by seeds is also dependent on the temperature. In many species of crops the sensitivity of seeds to light may change in response to seasonal temperature cycles (Batlla and Benech-Arnold 2007; Batlla *et al*. 2007).

Temperature and water mainly drive the rate of seed germination when aeration is not restrictive for non-dormant seeds (Probert 2000). It is controls seed germination in the nature, and information on cardinal temperatures is important for understanding the occurrence of plant species (Labouriau and Agudo 1987; cited by Leite and Takaki 2001; Godoi and Takaki 2004).The effect of temperature on elongation of the radicle and shoot, and thus, seedling emergence very important and temperature also determines germination percentage and germination rate (Kebreaba and Murdoch 2000).

Maximal germination can occur over a range of temperatures and germination declines sharply on either side of the range (Kebreaba and Murdoch 2000; Probert 2000). Germination rate usually increases linearly with increasing temperature from a minimal or base temperature (Tb) up to an optimum and decreases linearly to a ceiling temperature (Bradford 2002; Adama *et al*. 2007).

An important constraint in determining the suitability of a crop for production in a new region is the range of temperatures necessary for germination and seedling growth. Cardinal temperatures (minimum, maximum, and optimum), exist in most species and ecotypes for seed germination (Bewley and Black 1994). As a way to approximate cardinal temperatures for germination and early seedling growth, measurements are typically conducted across a range of temperatures. Thus, seeds germinate in a well-defined temperature range and germination rate depends on temperature. Cardinal temperatures for

germination of most crop plants tend to be similar to those of normal vegetative growth (Sparks 2004; Adama *et al*. 2007).

Characteristics of the light environment that affect germination include length, quality and photon irradiance of the light reaching the seed (Casal and Sanchez 1998; Sullivan and Deng 2003). For many species from different habitats, responses to light are mediated by phytochrome perception of the light (Casal and Sanchez 1998). Germination response has been also shown to be affected by day length experienced by maternal plants (Munir *et al*. 2001; Kettenring *et al*. 2006).

Environmentally induced photosensitivity of seeds is often interpreted as an adaptation ensuring that seeds will germinate in sites in which the probability of seedling establishment is high. Such a mechanism would be particularly advantageous for species with small seeds whose seedlings cannot survive in established vegetation (Gross 1984).

1.7.3 Seed germination under different salinity and drought stress

Germination, emergence and early seedling growth are the most critical periods for a crop to obtain a good stand. Losses in plant density during this period cannot be compensated for and will cause an equivalent loss in production. Under saline and drought conditions, the crop generally encounters more problems during germination, emergence and early seedling growth than during later growth stages and may even fail to establish.

Stress and strain are fundamental physical concepts that can be applied to biological systems. Physical scientists define stress as a force per unit area applied to an object. Strain is a change in a dimension of an object developed in response to a stress (Hopkins and Huner 2003). In a biological system, any external constraint that limits productivity below the genetic potential of a plant would be considered a stress (Hopkins and Huner 2003).

Salinity is a major environmental factor limits growth and yield of crops around the world (Miled *et al*. 2000). It may have a greater effect during certain phases of plant's life cycle than others. Seedling establishment and floral development are often thought to be the most sensitive stages (Otte 2001).

There are at least three components of salt stress, caused primarily by NaCl, which affect seeds: osmotic stress, specific ion toxicity, and induced nutrient deficiency (Kingsbury and Epstein 1986; Shabala *et al*. 2005).

Total germination of many species may be more affected by low osmotic potential than by specific ion effects (Sosa *et al*. 2005; Song *et al*. 2005). However, radicle growth may be strongly inhibited by specific ions. Successful seedling establishment depends on the frequency and the amount of precipitation as well as on the ability of the seed species to germinate and grow while soil moisture and osmotic potentials decrease (Tobe *et al*. 2005; Jamil *et al*. 2006).

1.7.4 Growth and development

Growth is the irreversible increase in size of plant and increase in size of organs. Growth is the accumulation of dry matter, firstly as sugars and then as structural and storage materials in leaves, stems, roots and grains in pods or fruits. Development determines the progress of changing a plant from one growth stage to other stages of its life cycle. The stages of development often need to be quantified and more precisely defined. The interaction between development and growth at each stage builds up the potential, and then the actual yield of the crop (Mendham and Salisbury 1995; Khattak and Pearson 2005).

The growth and development of plants is dependent on abiotic and biotic factors. Abiotic factors include the physical environmental conditions and biotic factors include animals, insects, and

diseases. Continued development and growth of the plant following successful germination are dictated by external environmental factors such as photoperiod, temperature, nutrient supply and water and by complex interactions with endogenous growth regulators that are collectively called the plant hormones or phytohormones including GA, ABA, cytokinin, brassinosteriods and auxin (Mendham and Salisbury 1995; Yang *et al*. 1996; Gray *et al*. 1998; Thingnaes *et al*. 2003; Zhao *et al*. 2003; Heggie and Halliday 2005).

Seedling development is also partly determined by photoreception and circadian clock genes. Because seeds often germinate in the dark, seedlings switch from heterotrophic to photoautotrophic growth when they reach favorable light conditions. Thus seedlings follow two developmental programs depending on light conditions. In darkness, seedling hypocotyls elongate (etiolation), while in light, hypocotyl elongation is inhibited (de-etiolation), cotyledons expand, and chloroplasts are developed (Stenoien *et al*. 2002).

Plants are influenced by sunlight reduction in a different ways. The main limitation of leaf net photosynthetic carbon assimilation at high photon flux density is the concentration of CO_2. When photon flux density decreases to approximately 40% of that a full sunlight, then carbon assimilation becomes light-limited (Cohen *et al*. 2005). However, plants have considerable ability to acclimatize to different light regimes through changes in leaf properties, as well as canopy structure (Syvertsen and Smith 1984). Their responses to light include a variety of adaptations at physiological and biochemical levels like alteration of growth rate and plant architecture on morphological characteristics and distribution and also the architecture of plant canopy influences the interception, absorption and scattering of solar radiation as passes through the atmosphere of earth to the soil surface (Faravani and Baki 2007; Nasrullahzadeh *et al*. 2007).

Expansion of leaf area is one of the fundamental processes of plant growth. Leaf area growth of a whole plant can be categorized into the initiation and enlargement of individual leaves. Leaf

growth pattern is known to be affected by various environmental factors such as temperature, water availability, irradiance, mineral nutrition and atmospheric CO_2 concentration. Generally, increased resource availability produces larger number of leaves and final leaf area in various plant species (Hocking and Mason 1993). In addition to environmental factors, leaf position along the stem (leaf order) affects leaf growth (Hocking and Mason 1993).

Light is one of the most important environmental factors with the high effect on regulating growth and development of plants. Light represents a source of energy when absorbed by chlorophyll and controls plant photomorphogenesis when absorbed by phytochrome. Phytochrome exists under a red (Pr) and a far-red (Pfr) absorbing distinct form. Upon exposures to red light, Pr undergoes photochemical conversion to a physiologically active far-red absorbing form, Pfr, which triggers a variety of complex physiological responses such as flowering and seed germination (Mancinelli 1994; Leite and Takaki 2001).

Plants change of their growth pattern to adapt themselves to the environment when they encounter to environmental stimulant. Solar radiation has a high impact on crop growth, development, survival, reproduction and yield. Some plants are capable of acclimating to varied light conditions through plasticity in leaf morphology and physiology with some of them showing the typical sun-shade morphological responses including decreasing stomatal densities, increasing specific leaf area, and increasing leaf area ratio with decreasing light levels in different light conditions (Boardman 1977; Aleric and Kirkman 2005).

During the cloudy days and rainy season, photosynthesis is generally decreased because of reduction in light intensity and duration brought about by excessively cloudy weather (Singh, 2000; Chaves *et al*. 2007). Shade plants acclimated to low irradiance with an anatomical and physiological change leading to photosynthetic characteristics different from sun plants (Boardman 1977; Oguchi *et al*. 2003).

Results of different studies have shown that the manipulation of spectral quality has potential for growth control in a range of ornamental plants similary the growth response of crops to light spectral quality can be exploited to deliver a range of agronomically desirable plant traits (Haeringen *et al*. 1998; Khattak and Pearson 2005; Paul and Moore 2006).

When plants are grown at higher temperature at the night and day, plant height will be reduced, flowering is delayed and leaf chlorosis set in. It limits the use of temperature manipulation for plant growth regulation (Karlsson *et al*. 1989). In many crop plants uniformity, stem and internode elongation are important characteristics. Especially in the horticulture, the temperature and duration of the photoperiod (altering day and night length) of the plants are manipulated routinely (Grindal *et al*. 1998).

Rapid root growth after establishment consists of the vertical extension of a taproot, lateral growth of secondary roots and then deposition of reserves principally in the taproot. Root distribution is close to the maximum at the end of flowering so earlier flowering of *B. rapa* L. lacks an extensive root system (Richards and Thurling 1979). On less compacted soils however, direct drilling gave a well-developed and well-distributed root system with depth (Gregory 1998).

The growing point of the plant produces leaf initials in a helical arrangement with a phyllotaxy of about 130 between leaves. As the early growth of the *Brassica* plant is very much dependent on temperature and photoperiod, these factors have a large effect on leaf initiation, appearance and expansion (Mendham and Salisbury 1995; Nanda *et al*. 1996). The environmental parameters that determine final leaf number are most likely to be those triggering the plant from vegetative to floral development. Nanda *et al.* (1995) observed a reduction in final leaf number under long photoperiods and lower temperatures. Leaf appearance of *B. napus* was more sensitive to weather parameters than *B. juncea.* They also studied the effects of temperature and photoperiod on the rate of leaf appearance in *Brassica* species in India and observed rapid development of leaves

with long of the photoperiod. Appearance of the first leaf was delayed 1.35 days for each 1°C reduction in mean temperature. The leaf number at any time after the appearance of leaf one, would be equally well predicted by using a relationship based on leaves per day as well as on leaves per degree day (Nanda *et al*. 1995; Razzaque *et al*. 2007).

Controlled environmental studies on leaf expansion and duration (Morrison *et al*. 1992) showed that leaf area development of *B. napus* cultivar "Westar" followed logistic shape growth functions. There is evidence for an effect of temperature and photoperiod on leaf area development. Photoperiod influences the reproductive development of crops and rapid development of the reproductive phases will limit leaf initiation in crops with a terminal raceme such as canola and mustard. Depending on the photoperiodic response, crops develop lower number of leaves (Morrison *et al*. 1992; Robertson *et al*. 1999).

Higher leaf area indices (LAI) can be achieved by either faster leaf expansion rate or longer periods between sowing and flowering. However, the advantage of using later flowering cultivars can only be achieved in a long growing season (Sidlauskas and Rife 1999; Sidlauskas and Bernotas 2003; Sidlauskas and Tarakanovas 2005).

The first sign of flowering commences on the main stem, which becomes the terminal inflorescence or raceme. Once the main stem flower buds are formed, axillary buds lower down will then begin to develop sequentially into the primary branches in a basipetal direction (Diepenbrock 2000; Razzaque *et al*. 2007). In general, first flowering occurs in the first primary branch and sequence downwards.

Several *Brassica* species have been shown to flower earlier after exposure to lower temperatures. European winter cultivars required vernalization before flowering. In most other cultivars, either vernalization or long days were necessary for prompt flowering, with only a few

lines such as very early Indian *B. rapa* showing little response to either (Myers *et al*. 1982; Norton *et al*. 2004). Australian cultivars were intermediate and Canadian cultivars were least responsive (Farré *et al*. 2002; Robertson *et al*. 2002).

Photoperiod response of *B. napus* and *B. juncea* occurs between 10.8 and 16.3 hours (Robertson *et al*. 2002). Nanda *et al*. (1996) found that the greatest response occurred between 12 and 14 hours. On average a change in photoperiod from 12 to 14 hours reduced the time to flowering by 40 %. Temperature and photoperiod can interact very strongly to change phenological development in many crops. High temperatures delay development at short photopenods, but accelerate it at long photoperiods. Hence, the duration taken to complete a phenological phase is better expressed by thermal time above a base, as it masks any differential effect of temperature and exposes and highlights the effects of photoperiod (Nanda *et al*. 1996). Late flowering genotypes respond more to vernalization and photoperiod than early flowering genotypes (Robertson *et al*. 2001; Robertson *et al*. 2002).

There is evidence of interaction effects of light intensity and temperature on plant development. The phase of emergence to flowering response to light intensity and a low light regime delayed flowering. Nanda *et al.* (1996) reported that low night temperatures also had an effect on the phenological development between plant emergence and bud development.

Pods commence rapid growth in length within a few days of anthesis, whereas rapid seed growth occurs after about 20 days. Pods reach full length before rapid seed growth start, and seeds attain 35 % of their final dry weight when the pod wall reaches full size and weight. The pod wall gains no more dry matter after dehydration commences, at 50 days from anthesis, whereas seeds increase dry matter by 42 % during their period of dehydration, from 50 to 75 days after anthesis. When rapid pod growth commences, stem and branch dry weight accumulation and extension are

close to their maximum, and leaf area is already declining rapidly (Hocking and Mason 1993; Robertson *et al*., 2002).

Water and nutrient stress either curtails flowering or limits success rate. A direct relationship was shown between the date of last nitrogen application and success rate of flowers (Lewis and Thurling 1994; Mendham and Salisbury 1995).

Seed survival until final harvest depends on factors such as supply of assimilates and water. Mendham and Salisbury (1995) observed the changes in number of seeds per pod with pod growth. From a consistent number of around 30 seeds per pod at flowering, the numbers of surviving seeds declined over a 3 weeks period, being stable in the last 3 to 4 weeks before maturity. Pods produced an average of 13, 11 and 8 seeds in upper, middle and lower sections of the canopy when sown early in the season. In the denser pod canopy of the early sowing, seed losses were greater and mainly in the lower levels of the canopy, whereas in the late sowing with less competition between pods, there was little difference in seed abortion between levels of the canopy.

The period over which seeds were lost coincided with the main growth of the pod walls and before the seeds themselves commenced rapid increase in dry weight. A study conducted under glasshouse conditions also revealed that the critical period for seed abortion is 2 to 3 weeks after flowering (Robertson *et al*. 2002). Similar results were found in *in vivo* observations of developing pods using X radiation and photography of developing pods (Pechan and Morgan 1985). Duration for seed growth was determined by Mendham and Salisbury (1995) as ranging from 35 to 55 days starting from 23 days after flowering. The rate of growth per seed, ranged from 0.08 to 0.12 mg/day and was a function of internal (crop size, leaf and other photo synthetic area, carbohydrate reserves) and external factors (radiation, water supply, temperature).

During seed development a number of quality changes occur before the final chemical composition of the mature seed is realized. At this time the rate of oil deposition follows a sigmoid curve. Oil concentration increased in a similar way to seed dry weight, reaching a maximum percentage after about 60 days, but the total oil concentration increased further with dry matter accumulation. Droplets of storage oil are first evident about 18 days after pollination. They increase in size and number between approximately 20 and 30 days after anthesis.

Oil concentration reaches a plateau at physiological maturity with little further change occurring until seed maturity (Saroop *et al*. 1998; Saroop *et al*. 1999). At seed maturity, about 80 % of the oil is concentrated in droplets in the cotyledonary cells. Oil concentration in the hypocotyl and radicle of mature seed is low while the seed coat contains only 7 to 12 %. Rapid nitrogen accumulation occurs in the early stages of seed development. Storage protein begins to accumulate when the embryo commences to grow rapidly to replace the endosperm and fill in the fully expanded seed coat. The onset of seed protein accumulation coincides with rapid cell expansion and rapid increase in embryo weight. Most of the proteins in mature seed are found in the cotyledons.

Among the environmental factors that regulate oil concentration, temperature has been found to be one of the most important, with high temperature reducing oil concentration (Hocking and Mason 1993). Irrigation can increase oil concentration (Al-Jaloud *et al*. 1996) while waterlogging and water stress reduce it (Mendham and Salisbury 1995). Hocking and Mason (1993) also reported the negative effects of drought and high temperature, that usually occurs during the later stage of crop growth in Mediterranean-type environments, on oil concentration of canola. High nitrogen fertility tends to reduce oil concentration (Al-Jaloud *et al*. 1996). Oil concentration can also be reduced when frost prematurely arrests seed development.

1.8 Intra-specific competition and self-thinning

The self-thinning rule is one of a complex of propositions about how plant stands grow in the absence of crowding-independent mortality (Westoby 1986). In plant populations, increase density can intensity plant-plant competition directly (Holm *et al*. 2007), and competition among plants is an important factor in affecting size hierarchies, growth rate, survivorship, and reproductive output (Weiner and Whigham 1988; Bonan, 1991; Weiner *et al.* 2001). Among these components, size hierarchies and reproductive output have been mostly investigated because they are of tremendous ecological and evolutionary significance.

The early study of intraspecific competition in plants was stimulated by the needs of foresters and agronomists to determine the optimum spacings of plants for maximizing yields. When crowding is sufficient to cause death, paths run from lower right (high density, low biomass) towards upper left (lower density, higher biomass) (Smart 1986; Westoby and Howell 1986).

The self-thinning line is a very robust pattern, which can be obtained in modeling studies by a variety of different mechanistic assumptions. The study of intraspecific competition is concerned with the response of the individual plant within the population and not the response of the total plant population. By understanding the factors limiting the growth of the individuals within the population, the response of the population as a whole can be deduced. This is termed the "law of constant final yield" (Woodward, 1987; Wieganda *et al*. 2008).

It has long been recognized that size inequality always increased with increasing density (Weiner and Whingham 1988). The increase of size inequality may affect total reproductive output (Weiner *et al*. 2001). Weiner and Whingham (1988) presented a simple linear model of size-dependent reproductive output to explain the decrease in reproductive allocation (RA) in plants grown at high densities. It has also been reported that, in water deficits and mulching with clear

plastic film conditions in spring wheat (*Triticum aestivum*) populations, there is a negative correlation between RA and Gini coefficient. It was suspected that this negative correlation would exist along an elevated intraspecific competition gradient.

The self-thinning rule is widely accepted as an empirical generalization and quantitative rule that applies but the evidence supporting it has recently come under critical scrutiny. The theoretical and empirical bases for the density–mass boundary have been questioned (Morris 1999; Li *et al*. 2000).

1.9 Soil fertility and fertilizer augmentation on nutritional status

Availability of N, P and K in soil is often too low for economic yield of agricultural crops. Addition of fertilizers to maintain good fertility level is a standard practice in agricultural soils. However, excessive application of fertilizers, particularly N and K, due to their high solubility may result in a salinity build up in the soil. This is typically reflected by a decline in yield when the fertilizer levels exceed the optimum (Russel 1998).

Potassium is an essential element for all living organisms. Potassium is often considered to be a nutrient of primary importance for oilseed rape and Indian mustard. The role of K^+ in oilseed rape, as in other crops, is mainly to activate a wide range of enzyme systems. The range of physiological functions controlled by K^+ is very wide (Evans and Sorger 1966 cited by Pettigrew 1999) such as the opening and closing of stomata, formation of chloroplasts, transport of photosynthates, carbohydrate and nitrogen metabolism. K+ is present in an unbound form in the cytoplasm and does not enter into the composition of the structural or storage components or plants (Mengel and Kirkby 2004).

Potassium application has no effect on the number of seeds per pod but it tended to reduce 1000-seed weight of oilseed crops. Hence the yield increase was essentially due to

an increase in the number of pods and increased branching of inflorescence. Potassium plays a major part in the enzyme system that controls the metabolism of photosynthesis and their conversion to oil. It does not usually have a major effect on the seed oil content. Potassium fertilizer has no influence on any aspect of fatty acid composition in any of the crop (Abd-El-Gawad *et al*. 1990). Similarly, there was no effect on protein of winter rape (Mengel and Kirkby 2004). Overall, it is concluded that K^+ has a negligible effect either beneficial or detrimental on the quality, including seed oil content in rapeseed.

Nitrogen has contributed an estimated 40 percent to the increases in per-capita food production in the past 50 years, although there are local and regional differences and varying efficiencies (Smil 2002).

A significant increase in growth rate of plant and yield has been observed with the application of nitrogen. There was a progressive increase in dry matter accumulation, leaf area index (LAI), leaf area duration (LAD) and crop growth rate (CGR) with increasing nitrogen rate from 0-80 kg ha^{-1} (Ozer 2003).

Response of Indian mustard varieties to different levels of N (0,25,50 and 100 kg ha^{-1}) showed all the yield attributes and seed yield ha^{-1} increased significantly by nitrogen application over control. The number of branches per plant, dry mater, seed, straw and oil yield ha^{-1} was increased significantly with increasing level of N. However, seed oil content was decreased while 1000-seed weight and number of seeds $siliqua^{-1}$ were not affected to a significant level. Split nitrogen application (100 kg ha^{-1} at sowing and 50 kg ha^{-1} at post-flowering) enhances substantially growth, photosynthesis, N assimilation and yield of mustard following defoliation (Lone and Khan 2007).

Abd-El-Gawad *et al*. (1990) determined the effect of NPK fertilization on the yield and yield components of rape and reported that although nitrogen application increased seed, straw and oil yield, seed weight per pod and seed index but seed oil concentration decreased with increasing N application. Increasing the P_2O_5 rate to 45 $kgha^{-1}$ increased yield components, seed and straw yield. However, K_2O application had no significant effect on seed yield components.

Plant height, number of branches per plant, number of pods per plant, number of seeds per pod, 1000-seed weight, seed and oil yield of Indian mustard was improved at 100 % recommended rates of NPK + 10 t ha^{-1} farmyard manure (FYM) compared with 100 % NPK rate (Mandal and Sinha 2004).

Application of nitrogen at 240 kg/ha increased total dry matter production and combine seed yield. Seed yield increased mainly due to greater number of pods on the terminal raceme and heavier seed weight whereas number of seeds per pod was not affected. Sulphur fertilizer application did not significantly increase plant dry matter production, number of seeds per pod or individual seed weight (Asare and Scarisbrick 1995).

A high rate of N application increases leaf area development improves leaf area duration (LAD) after flowering and increases overall crop assimilation, thus contributing to increased seed yield (Smith *et al.* 1988). Ibrahim *et al*. (1989) concluded that yield increased with rates of N up to 213 kg N ha^{-1}. High N applications were found to cause lodging? Nitrogen rates had significant effects on plant height, stem diameter, branch/plant and seed yield of *Brassica napus* (Al-Barrak 2006). Saleem *et al*. (2000) studied the effect of NPK application on the seed yield and oil contents of three raya (*B. juncea* L.) cultivars

and observed that although growth and yield parameters were increased none of the fertilizers affected the seed oil contents of all the three cultivars of raya (*B. juncea* L.).

1.10 Thesis structure

This section outlines the scope of work of the research program underlying and provides a conceptual framework of subsequent chapters of this thesis, along with a brief discussion of how these chapters are interrelated. The first chapter also includes a general introduction and brief an overview of experiments, treatments, and measurements. This thesis aims to find some information about the production ecology and agronomic characteristics of *B. juncea* var. Ensabi. Chapter 2 is mainly ecophysiologically oriented and looks at processes of seed germination under different abiotic conditions, allometry response and growth pattern, development and phenology of *B. juncea* var. Ensabi exposed to shade or full light regimes and compare growth stages of this plant with *B. napus*. Chapter 3 refers to the nutritional status and effects of different levels of N, P, and K on yield and yield components of *B. juncea* var. Ensabi. Chapter 4 dealt with the population structure of the plant and its influence on self-thinning of the plant. Chapter 5 presents influence of a spacing gradient on the growth of *B. juncea* var. Ensabi whilest Chapter 6 explain about the chemical nature and allelopathic effect of different extracts of *B. juncea* var. Ensabi. Finally the last chapter (chapter seven) gives a general discussion of the results obtained. This chapter synthesizes information from previous chapters discuss them.

1.11 Research objectives

The overall objective of this thesis was to study introgression of agronomic traits of *B. juncea*, and to determine its performance in seed germination, fertilizer uptake and its effects on the production ecology of *B. juncea* var. Ensabi. This research was also

conducted to determine general growth pattern and intra-competition to understand the mechanisms of competition at the individual plant level.

The other objective of the current research is to study its nutritional quality and allelopathy properties in order to evaluate the allelopathic potential of different parts of *B. juncea* var. Ensabi.

CHAPTER 2

SEED GERMINATION UNDER DIFFERENT CONDITIONS, ALLOMETRIC RESPONSE AND GROWTH PATTERNS OF *BRASSICA JUNCEA* VAR. ENSABI

2.1 INTRODUCTION

2.1.1 Seed germination and seedling growth

Information on seed biology is essential for understanding the process and patterns within a plant community, such as the establishment of plants, seed development and germination (Vázquez-Yanes and Orozco-Segovia 1993; Godoi and Takaki 2004). It is widely accepted that successful seedling establishment depends on a seed's ability to germinate (Baskin 2005).

Seed germination is an important stage in the life history of plant, affecting seedling development and survival, and population dynamics. It is a complex physiological process that is responsive to many environmental signals, including temperature, water potential, light and other factors (Bewley and Black 1994; Baskin and Baskin, 1998), each of these factors ought to prevail in adequate quantum in order to make seed germination possible (Egly 1995).

Seed germination can be divided into three phases, imbibition, increased metabolic activity, and initiation of growth, which loosely parallel the triphasic water uptake of dry mature seeds. Morphologically, initiation of growth corresponds to radicle emergence; subsequent growth is generally defined as seedling growth. By definition, germination incorporates those events that start with the uptake of water by the quiescent dry seed and terminate with the protrusion of the radicle and the elongation of the embryonic axis (Bewley 1997; Gallardo *et al.* 2001).

During germination of mustard and canola, a radicle emerges first and then the hypocotyl extends and cotyledons emerge. Radicle and shoot elongation, unlike seed germination, which is driven by temperature and water potential (Gummerson 1986), are mainly driven by temperature (Dahal and Bradford 1994).

2.1.1.1 Seed germination under different light and temperatures

Light is considered to be an important environmental factor which govern the germination of seeds in many plant species (Nabi 1999). Characteristics of the light environment that affect germination include length, quality and photon irradiance of the light reaching the seed. For many species from different habitats, responses to light are mediated by phytochrome perception of the light environment (Casal and Sanchez 1998; Vázquez-Yanes and Orozco-Segovia 1993; Lindig-Cisnero and Zedler 2001).

Temperature and water mainly control the rate of seed germination when aeration is not limiting for non-dormant seeds (Gummerson, 1986, Bradford, 2002). Temperature also plays a critical role in the elongation of the radicle and shoot, and thus, seedling emergence (Dahal and Bradford 1994; Roman *et al.* 2000). For most species, temperature determines germination percentage and germination rate (GR) (Ellis, 1992; Kebreaba and Murdoch, 2000). Cardinal temperatures, the minimum (T_b), the maximum (ceiling temperature, T_c) and optimum temperatures (T_o), exist in most species and ecotypes (Bewley and Black 1994) for seed germination. Maximum germination can occur over a range of temperatures and germination reduce sharply on either side of the range (Kebreaba and Murdoch 2000). Germination rate usually increases linearly with increasing temperature from a minimal or base temperature (T_b) up to an optimum and decreases linearly to a ceiling temperature (Bradford, 2002; Rowse and Finch-Savage 2003).

Thus, seeds germinate in a well-defined temperature range and germination rate depends on temperature. It is not surprising that germination response to temperature is related to ecological and geographical distribution of species and ecotypes (Probert 2000; Baskin and Baskin 2004), because germination is a critical stage of the life cycle reflecting adaptation to local habitats (Probert 2000).

Temperature has a primary influence on germination, affecting the capacity for germination by regulating dormancy and the rate or speed of germination in non-dormant seeds. It has been recognized since at least 1860 that three cardinal temperatures (minimum, optimum and maximum) describe the range of temperature over which seeds of a particular species can germinate (Bewley and Black 1994).

The cardinal temperatures for germination are generally related to the environmental range of adaptation of a given species and serve to match germination timing to favourable conditions for subsequent seedling growth and development (Alvardo and Bradford 2002). Smith (1982) reported the light requirement by seeds is also dependent on the temperature. Seeds of many plant species, mostly those that inhabit open or frequently disturbed habitats, require light to germinate. Environmentally induced photosensitivity of seeds is often interpreted as an adaptation ensuring that seeds will germinate in sites in which the probability of seedling establishment is high.

2.1.1.2 Seed germination under different salinity and drought stress

Stress and strain are fundamental physical concepts that can be applied to biological systems. Physical scientists define stress as a force per unit area applied to an object. Strain is a change in a dimension of an object developed in response to a stress (Hopkins and Huner 2003).

Salinity is an increasing environmental problem throughout the world. According the estimation of the United Nations Environment Program, 20% of cultivated land worldwide is adversely affected by a high salt concentration, which inhibits plant growth and yield (Megdiche *et al.* 2007). Salts in the soil water may inhibit plant growth for two reasons. First, the presence of salt in the soil solution reduces the ability of the plant to take up water, and this leads to reductions in the growth rate. This is referred to as the osmotic

or water-deficit effect of salinity. Second, if excessive amounts of salt enter the plant in the transpiration stream there will be injury to cells in the transpiring leaves and this may cause further reductions in growth. This is called the salt-specific or ion-excess effect of salinity (Greenway and Munns 1980; Yao and Fang 2009). The definition of salt tolerance is usually the percent biomass production in saline soil relative to plants in non-saline soil, after growth for an extended period of time. For slow-growing, long-lived, or uncultivated species it is often difficult to assess the reduction in biomass production, so percent survival is often used.

As salinity is often caused by rising water tables, it can be accompanied by waterlogging. Waterlogging itself inhibits plant growth and also reduces the ability of the roots to exclude salt, thus increasing the uptake rate of salt and its accumulation in shoots.

Plants can be divided into two broad groups with respect to salt tolerance: halophytes (salt tolerant) and glycophytes (salt intolerant). Halophytes can grow and complete their life cycle in saline environments, whereas glycophytes cannot. The responses of seeds of halophytes to salt differ from those of non-halophytes. Thus, compared with glycophytes, seeds of halophytes germinate at higher salinities, and they maintain viability even under extreme salinity or osmotic stress, recovering and germinating when the water potential of the medium increases (Ungar, 1995; Xiao *et al.* 2008). A survey of the light and temperature requirements for germination of 91 halophytes of salt marshes and salt deserts included information on the light/dark requirements for germination of 23 species and on the optimum temperature requirements for germination of all 91 species (Baskin &Baskin 1998). Seeds of four species required light for germination, four germinated at higher percentages in light than in dark, 13 germinated equally well in light and dark, and two germinated to higher percentages in dark than in light. The

optimum temperature for germination ranged from 5 to 35/25 ^{o}C, and the mean optimum was about 21 ^{o}C (Baskin and Baskin 1998).

The Indian mustard (*B. juncea* L.) is an important oil-yielding crop of saline lands, but information on salt tolerance, which indicates its salt-prone nature at the germinating stage (Kumar 1984). It has been shown to be more heat and drought tolerant than the other spices, with a range of contributing characters (Mendham and Salisbury 1995).

2.1.1.3 Effect of different light regimes on growth patterns

Plants show changing of growth pattern to respond to conditions in their external environment to adapt themselves to the environment. Light is one of the environmental factors that is known as the most important environmental factor regulating growth and development, ultimate energy source, a signal of direction and timing of plants. Light quality has been demonstrated to influence many aspects of plant growth and morphology (Smith 1982; Hoson 1999; Khattak and Parson 2005).

Plants are influenced by reduction of sunlight in different ways. The main limitation of leaf net photosynthetic carbon assimilation at high photon flux density is the concentration of CO_2. When photon flux density decreases to approximately 40% of full sunlight, then carbon assimilation becomes light-limited (Chelle 2006).

Continued development and growth of the plant following successful germination is dictated by external environmental factors such as light and temperature and by complex interactions with endogenous phytohormones including GA, ABA, cytokinin, brassinosteriods and auxin (Heggie and Halliday, 2005). Solar regimes controlled the rate of many of physiological and morphological development stages occurring in plants. Light controls plant photomorphogenesis, when absorb by phytochrome (Singh *et al.* 1996).

Intensity, duration and quality of light are important factors affecting plant growth (Soontornchainaksaenga *et al.* 2001). High light intensity substantially increased the total number of expanded leaves, dry matter, sugar content and nitrogen absorbed in *Phalenopsis* species (Kubota and Yoneda 1993). But, excessive light intensity causes stunting of the stem and leaf of alpine plants. High light intensity stimulated growth, tillering and yield per tiller and increased the stem proportion of *Brachiria bizantha* and *Panicum maximum*. It is greatly increased the number of sclerenchyma cells and their wall thickened in all organs (Deinum *et al.* 1996). Higher light intensity has more violet and ultra-violet radiation that cause the production of excess phenolic compounds in *Zosteria marina* (Vergeer *et al.* 1995). In cotton, leaf area was increased under low light intensity (Roussopolus *et al.* 1998). Most lower plants like mosses and ferns, as well as several woodland wild flowers are retarded in their growth or killed by high-sunlight intensity (Soontornchainaksaenga *et al.* 2001). Photoperiod with day length increase primarily altered phonological development of *Brassica* species.

Shade imposes a limitation to biological productivity in plants, although the extent of the limitation varies with shade tolerance of the species and the nitrogen supply (Wong 1991). Uniform high-nutrient conditions and completely predictable changes in light environment during ontogeny are common for crop plants, and are known to enhance genetic differentiation. Plants adjust their development in response to stressful conditions if the allocational pattern of underdeveloped plants is not consistent with optimal foraging behaviour. Interestingly, variation in specific leaf mass, the trait considered important in adaptive shade-avoidance responses, is partially attributable to ontogenetic plasticity (Semchenko and Zobel 2005).

2.1.1.4 Growth and development stages

It is important to understand how an Ensabi plant grows and how growth can be affected by different management to make effective managements decisions. Management timing of use application based on growth stages of a crop, can improve of the efficiency of the inputs.

Phenology defined as the study of biological events that occur once in the growing stages of the life of a crop. It describes, and measures developmental and physiological processes controlling growth and development, and the environmental influences (Broadhead *et al.* 2003).

Development is the progress of a plant through the stages of its life cycle and 'growth' is the increase in size of organs, and the accumulation of dry matter, firstly as sugars and then as structural and storage materials in leaves, stems, roots, grains, pod and fruits (Mendham and Salisbury 1995). The stages of development are often needed to be quantified and defined. The interaction between development and growth at each stage builds up the potential, and the realization of the potential yield. The growth and development of *B. juncea* plant is continuous but can be divided into easily recognizable growth stages. To define and quantify the stages of development numerical keys are very important. Much of the published work on crop physiology of *Brassicas* has been carried out on *B. napus.* The first key that widely used to define and quantify the stages of development of *B. napus* was developed in Canada . In 1984, some phenological and development stages (**Table 2.1**) was developed in the United Kingdom (Sylvester-Bradley and Makepeace 1984). However, no keys have been developed specifically for *B. juncea.* Since, both species progress through similar developmental stages, the same keys could be used to define and quantify the stages of development of *B. juncea.*

This chapter focuses on the results of the study on the response of *B. juncea* var. Ensabi to environmental stimuli or factors and their interactions (e.g. temperature, light, drought stress, salinity, etc) on seed germination and seedling growth, reproductive growth and the phenology of *B. juncea* var. Ensabi, using growth regression models.

Table 2.1. Definition and codes for stages of development in oilseed rape (*Brassica napus*).

Definition	Code
Germination and emergence	
Dry seed	0.0
Imbibed seed	0.2
Radicle emerged	0.4
Hypocotyl extending	0.6
Cotyledons emerged	0.8
Leaf production	
Both cotyledons unfolded and green	1.00
First true leaf exposed	1.01
Second true leaf exposed	1.02
……	.....
Tenth true leaf exposed	1.10
Twentieth true leaf exposed	1.20
Stem extension	
No internodes detectable ('rosette')	2.00
One internode detectable	2.01
Two internodes detectable	2.02
……	……
Ten internodes detectable	2.10
Twenty internodes detectable	2.20

Table 2.1. (*Continued*)

Definition	Code
Flower bud development	
Only leaf buds present	3.0
Flower buds present but enclosed by leaves	3.1
Flower buds visible from above ('green bud')	3.3
Flower buds raised above leaves	3.5
First flower stalks extending	3.6
First flower buds yellow ('yellow bud')	3.7
More than half flower buds on raceme yellow	3.9
Flowering	
First flowers opened	4.1
20% of all buds on raceme flowering or flowered	4.2
30% of all buds on raceme flowering or flowered	4.3
40% of all buds on raceme flowering or flowered	4.4
50% of all buds on raceme flowering or flowered	4.5
60% of all buds on raceme flowering or flowered	4.6
70% of all buds on raceme flowering or flowered	4.7
80% of all buds on raceme flowering or flowered	4.8
All visible buds on raceme finished flowering	4.9
Pod development	
Lowest pods more than 2 cm long	5.1
20% potential pods on raceme more than 2 cm long	5.2
30% potential pods on raceme more than 2 cm long	5.3
40%) potential pods on raceme more than 2 cm long	5.4
50% potential pods on raceme more than 2 cm long	5.5

Table 2.1. (*Continued***)**

Definition	Code
Pod development(continued)	
60% potential pods on raceme more than 2 cm long	5.6
70% potential pods on raceme more than 2 cm long	5.7
80% potential pods on raceme more than 2 cm long	5.8
All potential pods on raceme more than 2 cm long	5.9
Seed development	
Seeds present	6.1
Most seeds translucent but full size	6.2
Most seeds green	6.3
Most seeds green brown mottled	6.4
Most seeds brown	6.5
Most seeds dark brown	6.6
Most seeds black but soft	6.7
Most seeds black and hard	6.8

2.2 MATERIALS AND METHODS

2.2.1 Experiment 1. Effect of light, temperature and different media on laboratory seed germination in *B. juncea* var. Ensabi

Germination experiments were conducted in the laboratories of Institute of Biological Sciences, University of Malaya (3^o 8' N; 101^o 42' E), Malaysia. For seed germination the fresh seeds of *B. juncea* were dried in an oven at 35 ^oC for a week and stored in a refrigerator at 5 ^oC until use. Fifty seeds were placed in each petri dish for each treatment, lined with 9 cm diameter Whatman No.2 filter paper. The filter papers were moistened with 6 ml of distiled water or other solutions of the chemical media H_2O, KNO_3, H_2O_2 and HNO_3. Untreated seed (H_2O, control pH=7), Seeds soaked in H_2O + 0.2 M KNO_3 (pH=2.5), seeds soaked in H_2O + 5% H_2O_2 (pH=2.8), seeds soaked in H_2O + 0.01 M HNO_3 (pH=2.5) and the same method was used for oven dried seeds. Petri dishes were placed at different temperature regimes of 15^o, 20^o, 25^o, 30^o and 35 ^oC under a photoperiod of two light levels (12: 12 h light: dark period and 24-h dark environment).

Similarly, another set of Petri dishes incubated in a double layer of aluminum foil envelops for dark treatment under the same condition.

Germination was recorded everyday and the germination percentage was noted 10 days after sowing. The data on germination were analyzed as germination percentages [(number of seedling/number of seeds) ×100]. For evaluation, the standard procedures for testing seed viability and growth recommended by ISTA (1985) was done for non germinated seeds. On the purpose of this study, germination was considered as being complete when the radicle emerged from the seed and at least 2-mm long. Germinated seeds were counted daily until germination stopped and the germination percentage and rate were estimated.

We performed an arcsine transformation to the percentage data, before statistical analysis to ensure homogeneity of variance. The effect of light, temperature and seed treatments on the germination and rate of germination were examined using analysis of variance (ANOVA).

2.2.2 Experiment 2. Effect of NaCl and polyethylene glycol (PEG 6000) on germination and seedling growth of *B. juncea* var. Ensabi

Two studies were separately conducted in the laboratories of Institute of Biological Sciences, University of Malaya, Kuala Lumpur (3^o 8' N; 101^o 42' E), Malaysia.

2.2.2.1 *Drought stress experiment*

Germination and early seedling growth (10 days) of *B. juncea* var. Ensabi was studied in an experiment using distilled water (control) and osmotic potentials (-0.20, -0.40, -0.60, -0.80, -1 and -1.2 MPa), which were prepared by adding polyethylene glycol (PEG 6000) to distilled water according to Michel and Kaufman (1983) to have the osmotic potential in PEG.

Mature, healthy and equal sized seeds of Ensabi were previously disinfected by immersion in a calcium hypochlorite solution, containing 5 % active chlorine, for one minute. The seeds were then washed three times with sterilized distilled water. Seed germination tests were carried out in sterilized 9 cm Petri dishes (that had been autoclaved for four hours) with Whatman No.1 filter paper. Each dish was moistened with the appropriate osmotic solutions (PEG-6000 solutions, osmotic potentials of -0.20, -0.40, -0.60, -0.80, -1 and -1.2 MPa) or distilled water for 0 MPa as a control. Germination tests were carried out in a growth chamber (Shel Lab, Model 2015-2E) at 20 ^{o}C, 25 ^{o}C and 30^{o}C.

Same appropriate solutions were added daily to each petri dish. Seeds were considered germinated when the radicle emerged with at least 2-mm long. The number of

germinated seeds was recorded daily (germination rate), and the final germination percentage and rate were estimated.

The following parameters, previously reported by others such as Jefferson *et al*. (2003), were calculated for all four species:

a) Final germination (FG) %: The maximum average percentage of seeds that germinated during the experiment.

b) Mean period of final germination (MPFG) = $(\sum_{i=1}^{d} NiDi)/FG$

c) Rate of germination **(**RG**)** = $\sum_{i=1}^{d} \frac{Ni}{Di}$

d) Percentage inhibition or stimulation= (100- $\frac{FG\ in\ different\ solution\ (\%)}{FG\ in\ distilled\ water(\%)}$)

Where,

N = daily increase in seedling number

D = number of days from seed placement, the subscript ***i*** might be any integer value up through D.

At the end of eighth day, 5 seedlings were randomly selected and the root, shoot and seedling length were measured. Additionally weight of oven dried (70^{o}C for 48 hours) of root and shoot of seedlings were measured.

The experimental design with respect to three factors arranged in a completely randomized design with three replications of 25 seeds per replicate. The first factor (temperature) had three levels (20 ^{o}C, 25 ^{o}C and 30^{o}C), the second had seven levels (0, -0.20, -0.40, -0.60, -0.80, -1 and -1.2 MPa).

2.2.2.2 *Salinity stress experiment*

Salinity levels of 0, -0.2, -0.4, -0.6, -0.8, -1 and -1.2 MPa, were created using distilled water (control) and Nacl, according to Van't Hoff's equation (Lang 1967; Michel and Kaufman 1983; Salisbury and Ross 1996). In this experiment all measurements were similar to drought stress experiment.

Data analysis

Homogeneity of variances was tested; data not normally distributed were transformed (e.g. using arcsin$\sqrt{x + 0.5}$ for percentage data); retransformed data were presented in the results. Anova was performed on the data. Differences between means were determined using Tukey's compromise test.

Data for the final germination percentage was analyzed with two way analyses of variance (Anova), arcsine transformation was done if where possible.

2.2.3 Experiment 3. Effect of light regimes on growth patterns of *B. juncea* var. Ensabi

To study on growth and structural demography and to assess general growth patterns in relation to different light regimes of *B. juncea* var. Ensabi an experiment was conducted at Rimba Ilmu, University of Malaya, Kuala Lumpur (3^o 8' N; 101^o 42' E), Malaysia. The experiment was done in greenhouse with clay pots measuring 35 cm in diameter, and 40 cm high, previously filled with garden soils of Malacca series (Mca), (Paramananthan 2000). Soil samples were collected before planting and soil analysis on composite sample of collected soil, was done to know the nutrient status and physico-chemical characteristics of the soil, prior to experiment (**Table 2.2**).

Table 2.2. Physico-chemical characteristics of garden soils of Malacca series (Mca), Malaysia

pH	EC	SP (%)	T.N.V. (%)	N (%)	P (ppm)	K (ppm)	OM (%)	Sand (%)	Silt (%)	Clay (%)
5.39	0.93	28.97	0.54	0.21	47	69	2.21	68.00	17.00	15.00

The plants were divided into two groups: (1) outdoor (FEP = fully exposed to sunlight) (mean midday radiation of 1812 µmole photon m^{-2} s^{-1}) and (2) inside in insect-proof house (PEP = partially exposed to sunlight) with 12 hrs of natural sunlight (mean midday radiation of 384µmole photon m^{-2} s^{-1}) and mean temperatures of 33 ^{o}C (day) and 25 ^{o}C (night). In this experiment growth parameter was measured, for example: leaf modules / plant / every three days, branch modules / plant / every three days, bud and flowers / plant / every three days after budding, immature & mature pods / plant / every three days after starting of pod stage, seed number/ pod/ plant/mature plant, seed weight/ pod/ plant/mature plant, plant height, stem diameter and total dry matter.

The growth data were analyzed with t-test and regression analyses were performed where appropriate. The process of finding the best fit was done by Curve Expert 1.3 by comparing the data to each model to choose the best curve. The XY data can be modelled using a toolbox of linear regression or nonlinear regression models, nonlinear regression models, interpolation, or splines.

2.3 RESULTS AND DISCUSSION

2.3.1 Seed germination under different light and temperature

Brassica juncea var. Ensabi germination seeds under different temperature regimes, failed to germinate fresh and dry seeds under incubated on darkness and light regimes subjected to all treatment media, and also 2, 3, 5 – Triphenyl tetra-zolium chloride test showed the seed embryos were killed by chemical media. Temperature affected the percentage and mean rate of germination seeds of *B. juncea*, but there no differences were found between fresh and dry seed treatments under different temperatures (**Fig. 2.1**, **Table 2.3**).

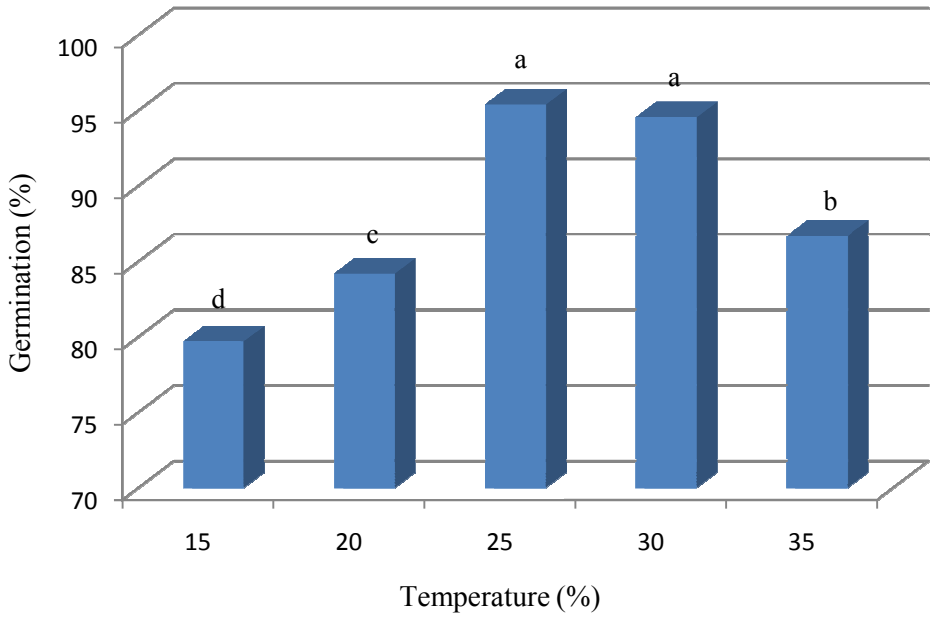

Fig. 2.1. Germination percentages of *Brassica juncea* var. Ensabi under different temperature regimes after 10 days. Figures followed by the same lower case letters are not significantly different (Tukey's HSD Post Hoc Test) (p<0.05).

Table 2.3. Analysis of variance for seed germination, lengths of shoot and root of seedlings of *Brassica juncea* var. Ensabi under different temperature and light regimes.

Source of variation	D.F	Mean square		
		Seed germination (%)	Plumule (mm)	Radicle (mm)
Temperature(^{o}C)(A)	4	850.11**	65.88**	160.79**
Seed treatment (B)	1	1.20	21.56**	25.25**
Light treatment (C)	1	3.20	4063.96*	29.79*
AB	4	6.01	4.86*	20.68**
AC	4	12.58*	11.78*	35.84**
BC	1	2.45	16.56*	41.44**
ABC	4	1.45	3.96*	6.73**
Error	60	0.98	0.37	0.25
CV %		12.53	14.94	15.74

F values * (p<0.05); ** (p < 0.01)

Measurable signs of germination occurred between 24 and 48 hours after sowing and at higher temperature germination occurred earlier. Most complete germination occurred at 25^{o}-30^{o}C and these temperatures were the best temperature for germination percentage (95.48% and 94.86% respectively) and 30^{o}C was the best temperature for

velocity (**Fig. 2.2** and **Fig. 2.3**). Válio and Scarpa (2001) reported that 91% of seeds of *C. hololeuca* germinated at a continuous temperature of 25°C at photoperiod of 12 hours.

Germination rate increased with temperature to an optimum of 30^{o}C and then decreased (**Fig. 2.3.**). There were also significant differences in germination between (15-20ºC) and (25^{o}-30^{o}C). Temperature had a major impact on seed germination and increase percentage and mean rate of some plant species (Benvenuti *et al.* 2001; Teketay 2002). Baki and Nabi (2003) reported Wrinklegrass was a positively photoplastic species, and seed germination was temperature-dependent and light-mediated, and Leite and Faravani and Baki (2007) reported that temperature strongly affected germination of *M. malabatricum* seeds and they showed that when temperature increased, germination velocity increased significantly.

Germination of coastal halophytic grasses from Pakistan was affected by different temperature under saline and nonsaline conditions. The optimal temperature regime for the germination of grasses was (20°- 30°C) studied both under light and dark conditions. At higher temperatures differences between light and dark germinated seeds were not significant (Khan and Ungar, 1999). No significant differences were observed between light and dark conditions on seed germination. Germination of African mustard seeds collected from southern Australia was not influenced by light conditions at the optimum temperature (Chauhan *et al.* 2006a), but seed germination of oriental mustard was stimulated by light, although, some germination occurred in the dark (Chauhan *et al.* 2006b).

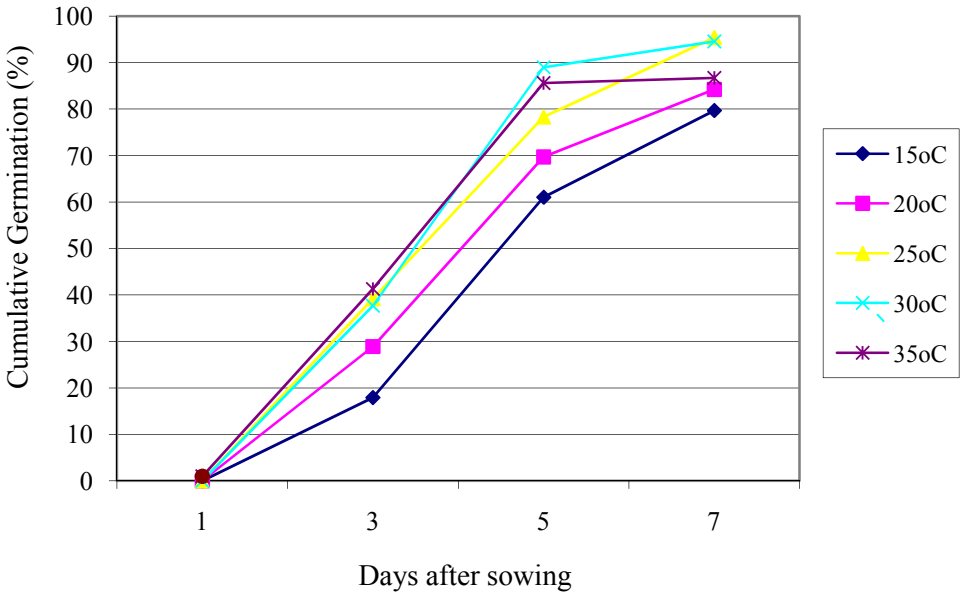

Fig. 2.2. Cumulative germination (%) at different temperatures and days after sowing in *Brassica juncea* var. Ensabi.

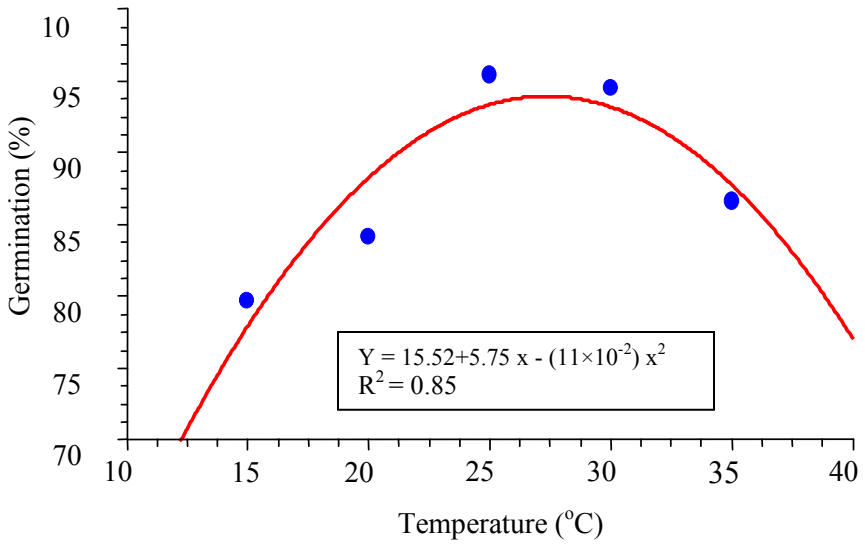

Fig. 2.3. Effect of temperature on seed germination (%) of *Brassica juncea* var. Ensabi.

The data relating to the growth of root seedling and hypocotyl after seven days of germination were shown in **Table 2.4**. Radicle and hypocotyl lengths of *B. juncea* var. Ensabi seeds were significantly at α=0.05 affected by temperature and light (**Fig. 2.4** and **2.5**).

Table 2.4. Comparison means of seed germination data of *Brassica juncea* var. Ensabi at different temperatures.

Temperature (^{o}C)	3 Days	5 Days	7 Days	Germination (%)	Shoot length (mm)	Root length (mm)	Shoot/ Root
15	17.9 d*	61.0 d	79.7 d	79.8 d	15.0 d	10.0 d	1.5 cd
20	29.8 c	69.7 c	84.2 c	84.2 c	17.5 c	11.3 c	1.6 c
25	39.2 b	78.3 b	95.5 a	95.5 a	22.2 a	15.2 a	1.5 d
30	37.7 b	89.0 a	94.6 a	94.6 a	21.7 ab	12.8 b	1.7 b
35	41.3 a	85.6 a	86.7 b	86.7 b	21.3 b	12.0 bc	1.8 a

* Those having the same letter (a,b,...) within each temperatures or seed treatments are not significantly different at $p<0.05$ by HSD test

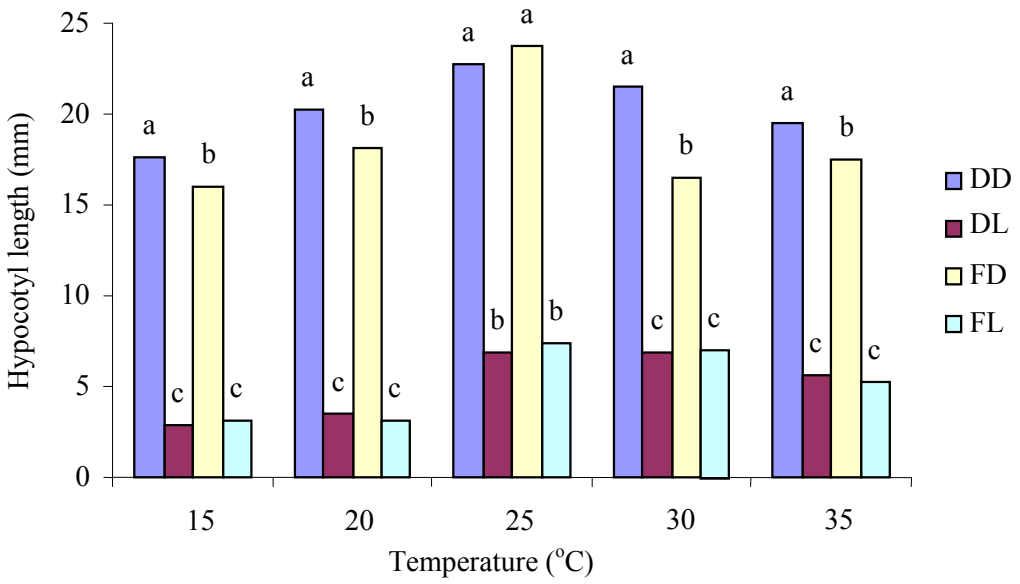

Fig. 2.4. Interaction effect of seed treatments (DD=dry seeds in dark condition, DL= dry seeds in light condition, FD= fresh seeds in dark condition and FL= fresh seeds in light condition) and temperatures on hypocotyle length of *Brassica juncea* var. Ensabi on different light conditions after 10 days.

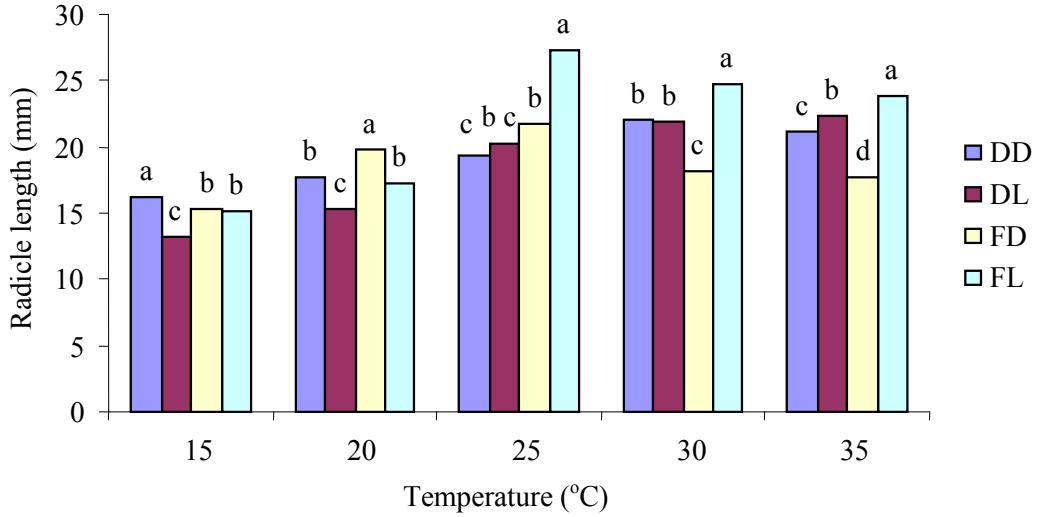

Fig. 2.5. Interaction effect of seed treatments (DD=dry seeds in dark condition, DL= dry seeds in light condition, FD= fresh seeds in dark condition and FL= fresh seeds in light condition) and temperatures on radicle length of *Brassica juncea* var. Ensabi on different light conditions after 10 days.

The hypocotyl indicated the optimum lenght growth was 22.8 (mm) at 25^{o}C on dry seeds under total darkness and for fresh seeds on same condition was 23.7 (mm) at 25^{o}C. The optimum hypocotyl length in light condition were 6.93 (mm) and 7.39 (mm) at 25^{o}C for dry and fresh seeds, respectively (**Fig. 2.6**). But the optimum linear growth of radicle measured 22.1 (mm) at 30^{o}C and21.7 (mm) at 25^{o}C under total darkness for dry and fresh seeds, respectively. The optimum seedling root length in light condition measured 22.3 (mm) and 27.3 (mm) at 35^{o}C and 25^{o}C for dry and fresh seeds respectively (**Fig. 2.7**). Characteristics of the light that affect germination include length, quality and photon irradiance of light reaching the seed (Casal *et al.,* 1998).

The growth of the hypocotyl was affected to a wide range in light condition and total darkness, although the radicle remained almost unaffected in both the conditions. It was concluded from these data that the hypocotyl growth was almost five times more in total darkness compared to its growth in light condition, whereas the growth of the radicle in light condition and darkness did not indicate much difference. The growth of the hypocotyl in total darkness also depended on a suitable temperature. Temperature has influenced to regulate the growth of hypocotyl in total darkness.

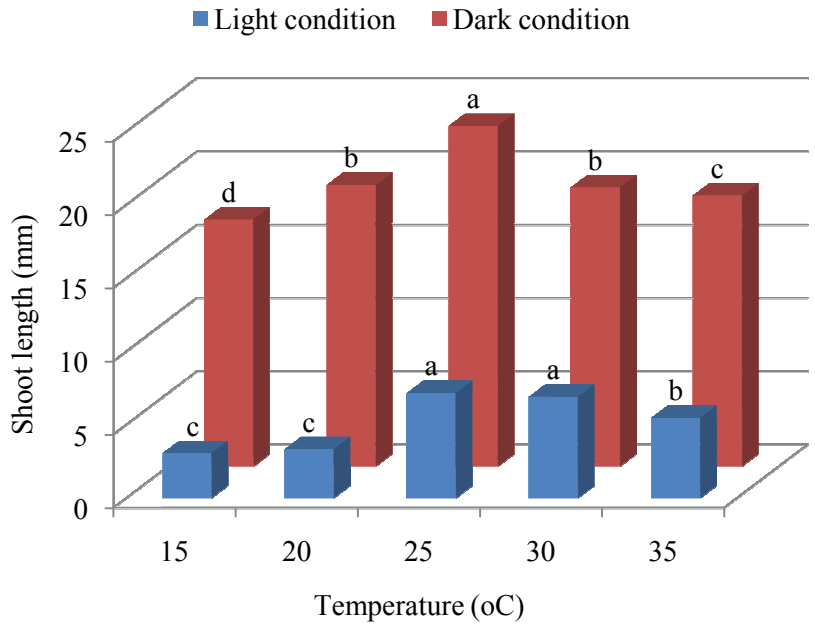

Fig. 2.6. Interaction effect of temperature and different light conditions on shoot length of *Brassica juncea* var. Ensabi seedlings of dried seeds. Figures followed by the same lower case letters are not significantly different (Tukey's HSD Post Hoc Test) (p<0.05).

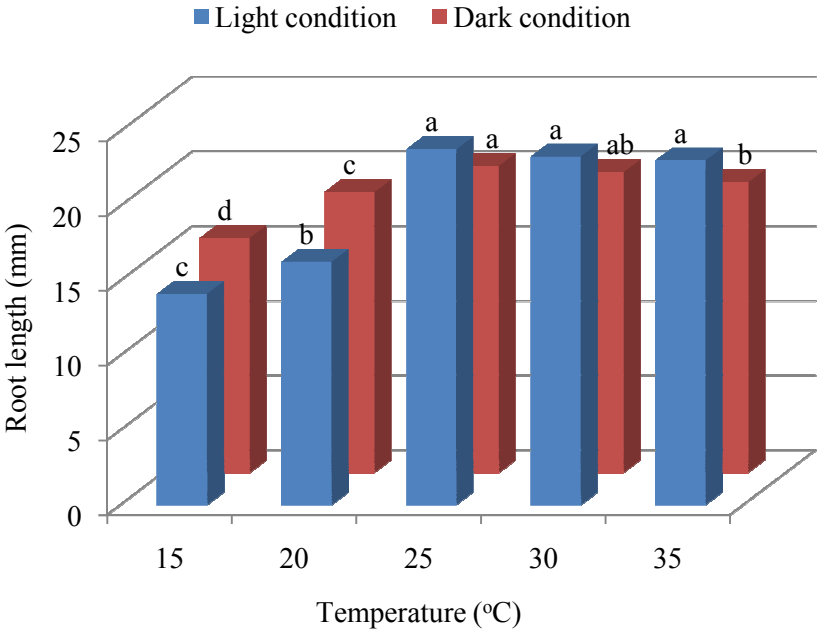

Fig. 2.7. Interaction effect of temperature and different light conditions on root length of *Brassica juncea* var. Ensabi seedlings of dried seeds.

2.3.2 Seed germination under salinity and drought stress conditions

Salinity significantly (p <0.05) affected the final germination rate and germination percentage of *B. juncea* var. Ensabi (**Table 2.5**, **Figs. 2.8** and **2.10**). Germination rate decreased with salinity or PEG 6000 osmotic solution (**Fig. 2.10**). These results were similar to those reported by Jeannette *et al*. (2002) and Okcu *et al.* (2005) who found that the mean time for germination of *Phaseolus* and *Brassica* species respectively, increased with NaCl and the increase was greater in higher concentration.

The highest percentage of seed germination recorded was in distilled water and germination percentages sharply decreased with increasing NaCl concentration. As the salinity level increased from 0 to10 MPa in NaCl, more than 80% reduction was observed in seed germination compared to control and germination was completely inhibited in higher salinities (**Fig. 2.8**).

It is also assumed that in addition to toxic effects of certain ions, higher concentration in salt reduces the water potential of the medium which hinders water absorption by germinating seeds and thus reduces germination (Jamil *et al.* 2006). It is suggested that the germination rate and the final seed germination decreased with the decrease of the water movement into the seeds during imbibitions (Jamil *et al.* 2005). Salinity stress can affect seed germination through osmotic effects. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity (Huang and Redmann 1995).

With PEG 6000, similar to NaCl concentration, seed germination and final germination rate of *B. juncea* var. Ensabi was significantly affected by PEG 6000 concentration (**Table 2.5, Figs. 2.9** and **2.10**). By increasing osmotic potential of PEG 6000, seed germination and final germination were decreased. In distilled water, percentage

of seed germination was highest. Higher amounts of PEG 6000 concentration in this research (-1.0 and -1.2 MPa) completely inhibited seed germination (**Fig. 2.9.**). Has been suggested that the first physiologic disorder, which takes place during germination, is the reduction in imbibitions of water by seeds which leads to a series of metabolic changes, including general reduction in hydrolysis and utilization of the seed reserve (Ahmad and Bano 1992). Salt and osmotic stress limit the mobilization of reserves in several species (Sidari *et al.* 2008).

Table 2.5. Changes in germination percentages of *Brassica juncea* var. Ensabi at different osmotic potentials of NaCl and PEG 6000.

Osmotic potentials	Germination percentage (%)	
MPa	PEG 6000	NaCl
0	96.67 a	96.67 a
-0.2	78.89 b	89.63 b
-0.4	50.74 c	78.89 c
-0.6	11.11 d	41.48 d
-0.8	2.22 e	34.07 e
-1.0	0.00 e	6.67 f
-1.2	0.00 e	0.00 g

* Values show the real germination percentages but variance analysis was performed using arcsine transformed values. Means followed by the same letter(s) were not significantly different at $P < 0.05$.

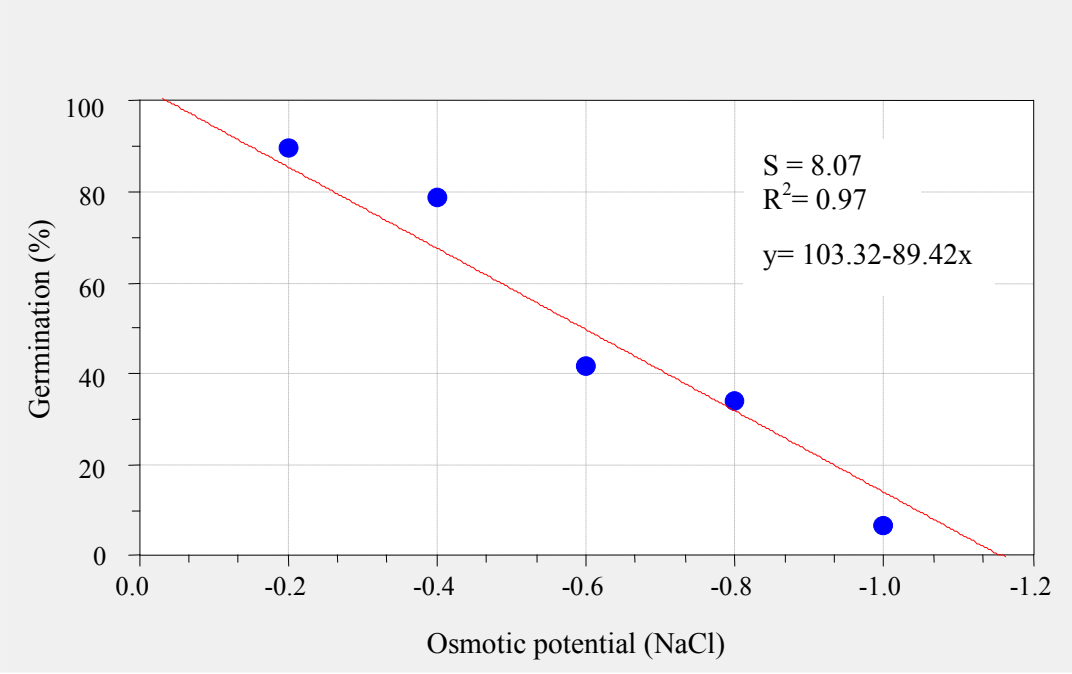

Fig. 2.8. Germination percentage of *Brassica juncea* var. Ensabi under decreasing external osmotic potentials created by NaCl.

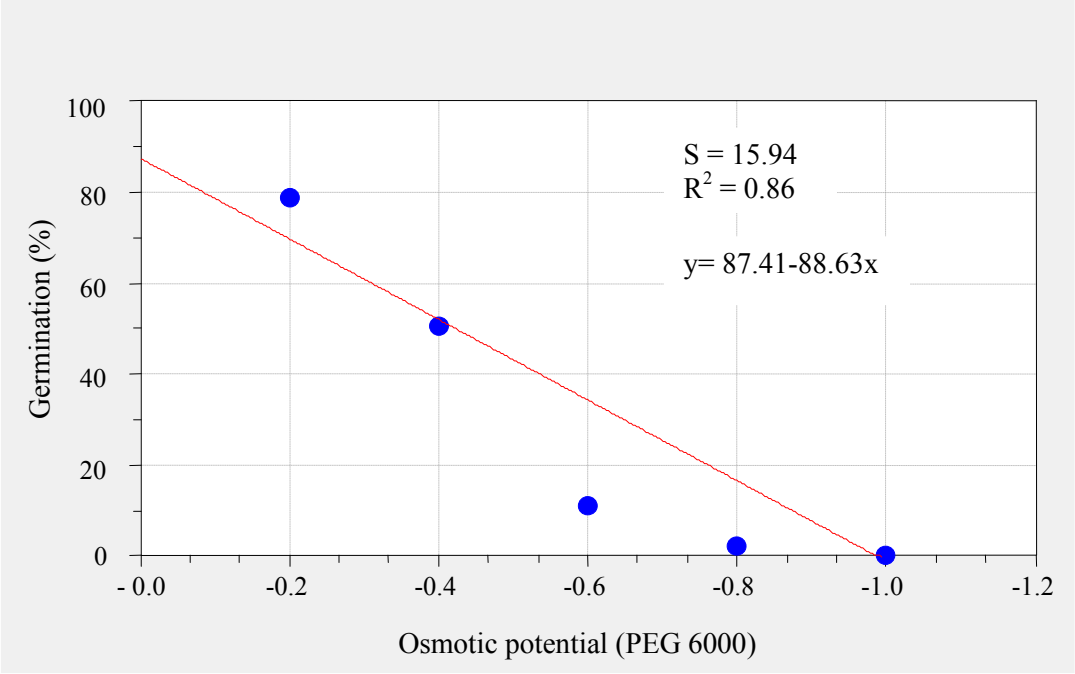

Fig. 2.9. Germination percentage of *Brassica juncea* var. Ensabi under decreasing external osmotic potentials created by PEG 6000.

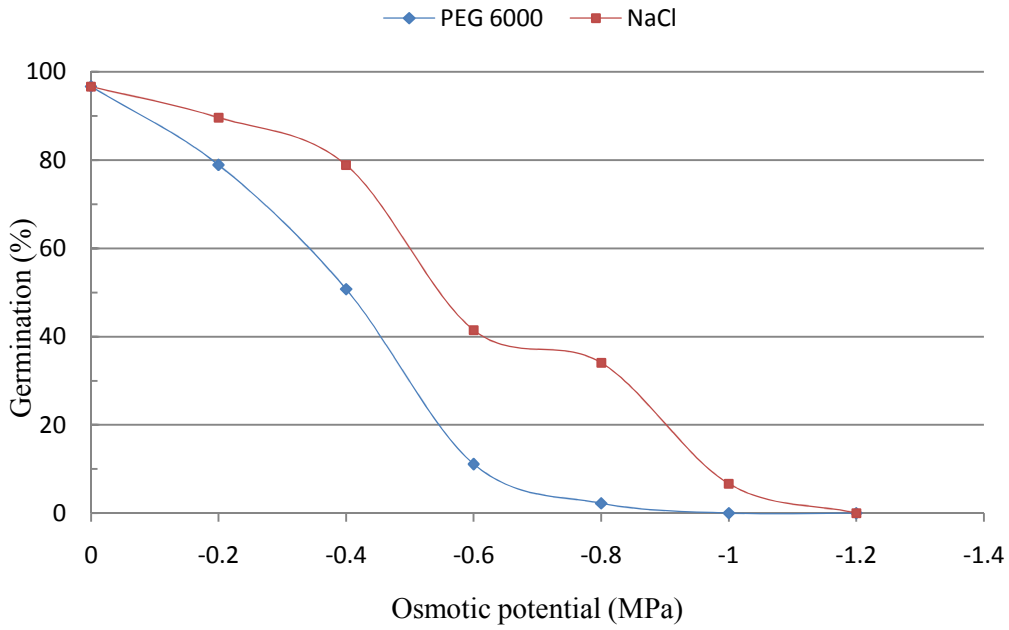

Fig. 2.10. Germination rate of *Brassica juncea* var. Ensabi under decreasing external osmotic potentials created by PEG 6000 or NaCl.

Effect of two regimes each of NaCl and osmotic (polyethylene glycol) stress on seed germination of 98 genotypes of *B. juncea* were investigated by Kuhad *et al.* (1989). Both stress types significantly reduced germination percentage, dry matter weight, shoot and root length of seedlings. In Indian mustard (*B. juncea*) increasing salinity levels of irrigation water progressively reduced seed germination of six cultivars (Ray and Khaddar 1990).

Signs of germination for control and low salinity and PEG 600 osmotic potentials treatments occurred between 24 and 48 hours after sowing but at higher salinity and PEG 6000 osmotic solutions it occured later (**Figs. 2.11** and **2.12**). Almost complete germination occurred at 25°-30°C and these temperatures were the best temperature for germination percentage and 30°C was the best temperature for the rate of germination.

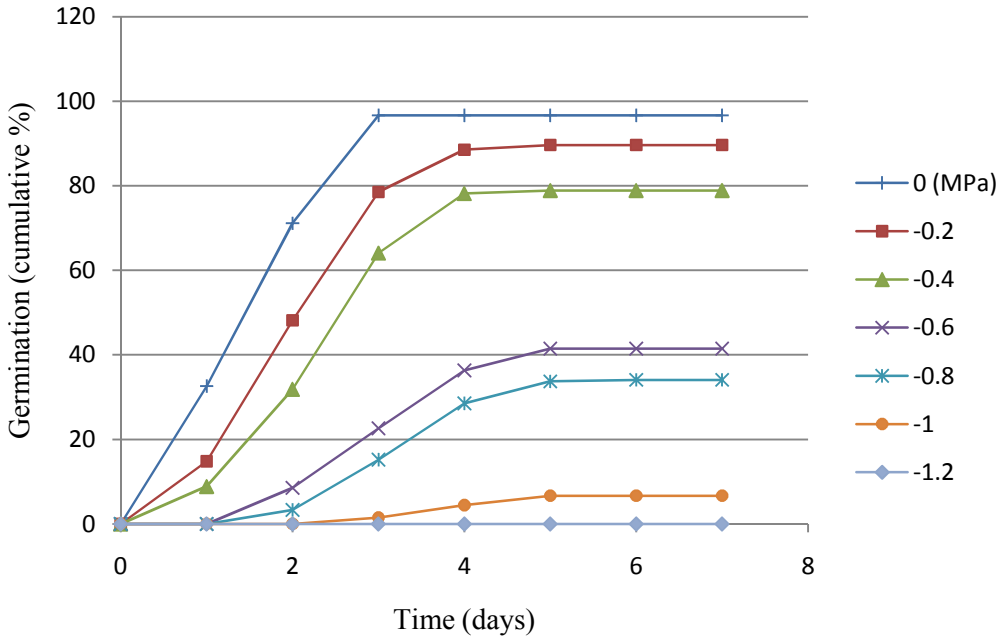

Fig. 2.11. Cumulative mean percentage germination of *Brassica juncea* var. Ensabi seeds against time and decreasing external osmotic potentials of NaCl.

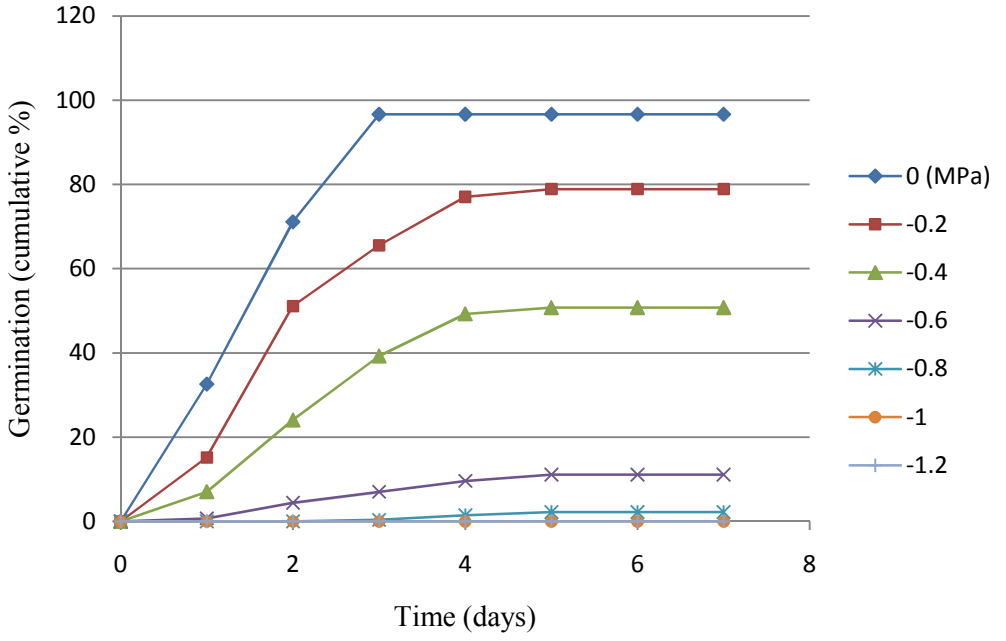

Fig. 2.12. Cumulative mean percentage germination of *Brassica juncea* var. Ensabi seeds against time and decreasing external osmotic potentials of PEG 6000.

Germination percentages improved with increasing in temperature under different salinity treatments or PEG 6000 osmotic solutions. Although we reported temperature significantly affected seeds germination of *B. juncea* var. Ensabi, there were no significant differences between different temperatures (Fallah Toosi and Baki 2007). Salinity and temperature interact in their control of seed germination of halophyte seeds (Khan *et al.* 2001). The adverse effect of high salinity is further aggravated by either an increase or decrease in temperature (Khan and Rizvi 1994; Khan 2002). Germination of many plants occurs at times when there is an optimal combination of day length, temperature regime, and salinity (Gutterman *et al.* 1995; Khan 2002). The results showed 20°C to be the optimal temperature for germination of Ensabi seeds in salinity and drought stresses situation and any increase or decrease in temperature inhibited germination. For this reason when temperature increased from 20^{o}C, there was no significant deference between different temperatures.

Seeds have the highest resistance to extreme environmental stresses, whereas germination is considered as the most sensitive stage and seedlings are most susceptible in the life cycle of a plant (Qu *et al.* 2007; Sidari *et al.* 2008). Therefore, successful establishment of a plant population is dependent on the adaptive aspects of seed germination and of early seedling growth (Qu *et al.* 2007).

Hypocotyl length was affected by NaCl and PEG 6000 osmotic stress but the effect of PEG 6000 was very evident (**Figs. 2.13** and **2.14**). Osmotic potentials of 8-10 MPa NaCl significantly decreased hypocotyl elongation ($P \leq 0.05$), whereas osmotic potentials of 12 MPa completely inhibited them ($P \leq 0.05$). No hypocotyl elongation occurred at NaCl concentrations of ≥10 MPa (**Fig. 2.13**). Parti *et al*. (2003) reported in *B. juncea* higher levels of salinity adversely affected plant growth, seed yield and total lipids of seeds.

In PEG 6000 solution, the hypocotyl lengths of seedlings decreased with an increase in water stress. Shoots elongation significantly decreased by osmotic potentials of 2-8 MPa. There was no hypocotyl elongation at osmotic potentials of 10 and 12 MPa and shoot elongation was completely inhibited (**Fig. 2.14**).

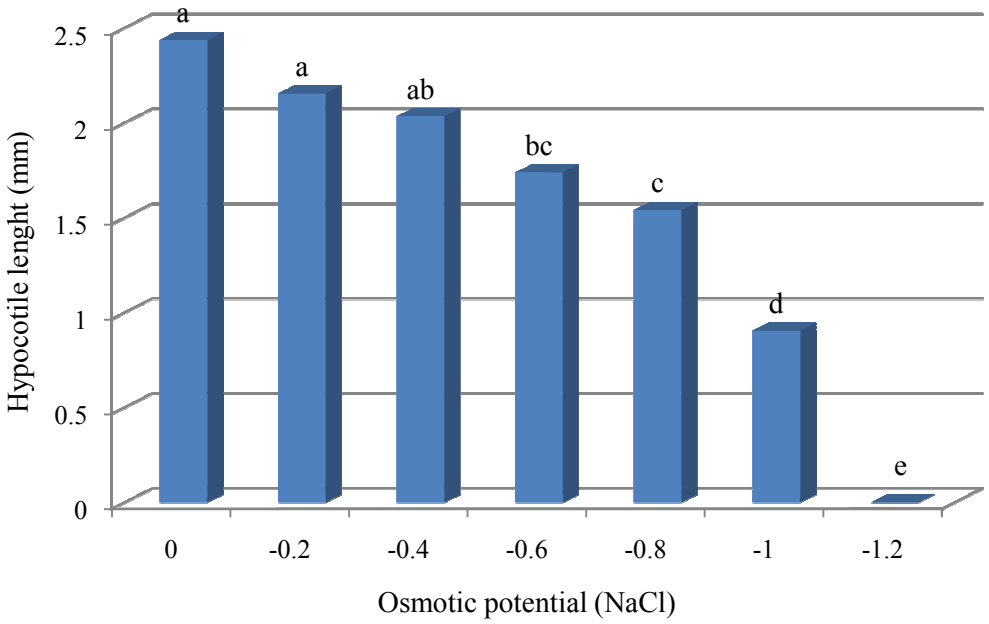

Fig. 2.13. Mean shoot length in solutions of increasing NaCl concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences ($p < 0.05$) between different osmotic potentials (by Tukey's test).

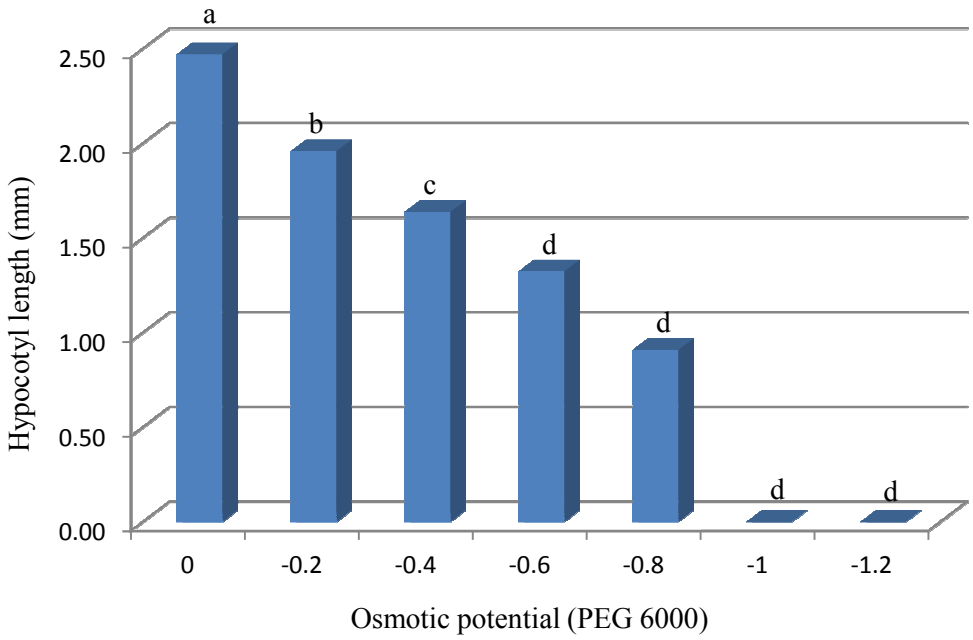

Fig. 2.14. Mean shoot length in solutions of increasing PEG 6000 concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences *(P* < 0.05) between different osmotic potentials (by Tukey's test).

Radicle elongation also significantly affected by NaCl osmotic stress and PEG 6000 solutions (**Figs. 2.15** and **2.16**). Results showed that NaCl concentration at 8 MPa stopped root growth stopped after seed germination and completely inhibited it at 10 and 12 MPa (**Fig. 2.15**). Reduction in root growth under saline conditions may either be due to decrease in the availability of water or increase in sodium chloride toxicity.

For PEG 6000 osmotic potentials it was completely different. Results showed that radicle growth was fast and at lower PEG osmotic potentials, radicle elongation significantly increased. Low osmotic stress (-2 and -4 MPa) improved the root length of *B. juncea* var. Ensabi. These concentrations of PEG osmotic potential exhibited longer seedling roots and radicles were significantly longer compared to the control. However radicle elongation declined by increasing concentration of the solution more than 4 MPa

and completely inhibited at 10 and 12 MPa. There were no significant differences between control and 6 MPa PEG solutions (**Fig. 2.16**). These results agree with those of Murillo-Amador *et al*. (2002) carried out on cowpea and results reported by Radhouane (2007) in pearl millet and Yagmur and Kaydan (2008) on triticale, who affirmed that a moderate and low osmotic stress (PEG or NaCl) improved root length of seedlings.

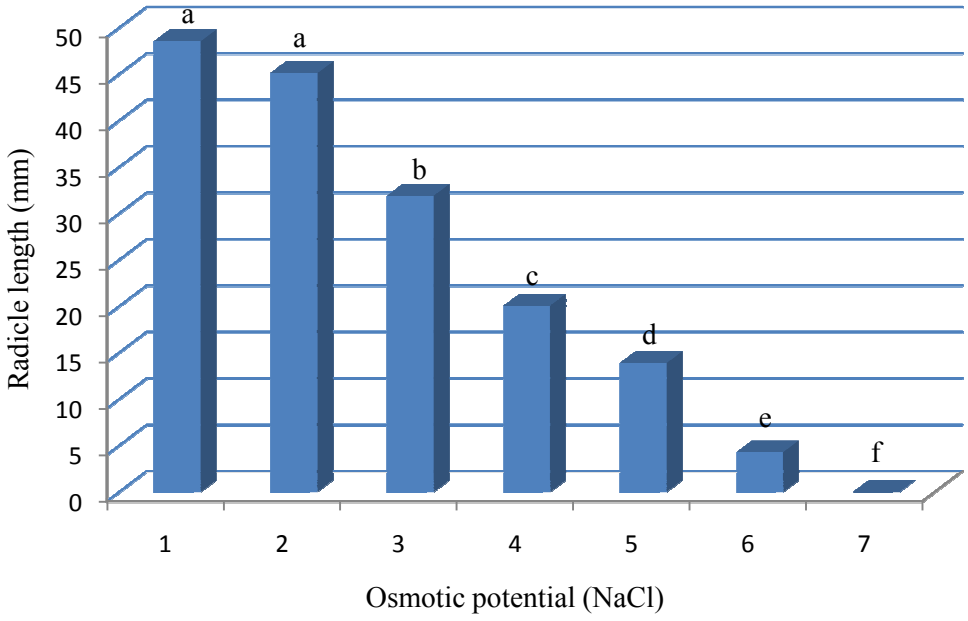

Fig. 2.15. Mean root length in solutions of increasing NaCl concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences *(P* < 0.05) between different osmotic potentials (by Tukey's test).

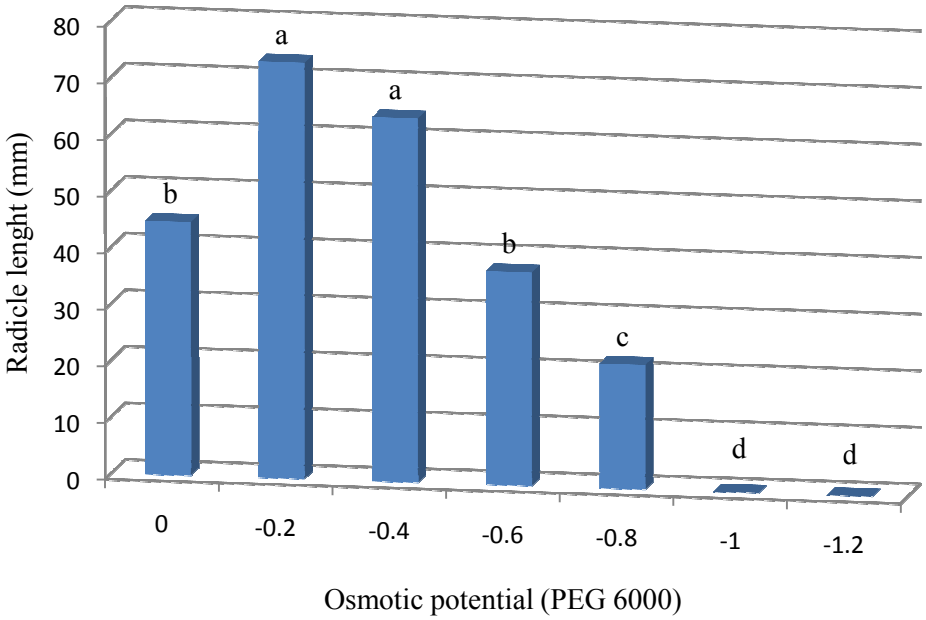

Fig. 2.16. Mean root length in solutions of increasing PEG 6000 concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences *(P* < 0.05) between different osmotic potentials (by Tukey's test).

The dry weights of hypocotyls were affected by NaCl solution. Results showed dry weights of hypocotyls increased initially with increasing salinity but this increase stopped at -2 Mpa. There were no significant different with the control at -6 MPa subsequently hypocotyl weight decreased sharply at osmotic potential of -8 and -10 MPa and was significant different from control (**Fig. 2.17**). Parti *et al*. (2003) in their research showed at low concentration salinity of NaCl, the plant growth seems to be normal, seed yield and total lipids were maximum.

Results also showed the growth of hypocotyl was inhibited by increasing PEG 6000 solutions. It was not significantly different between the control and moderate osmotic potentials of PEG (**Fig. 2.18**).

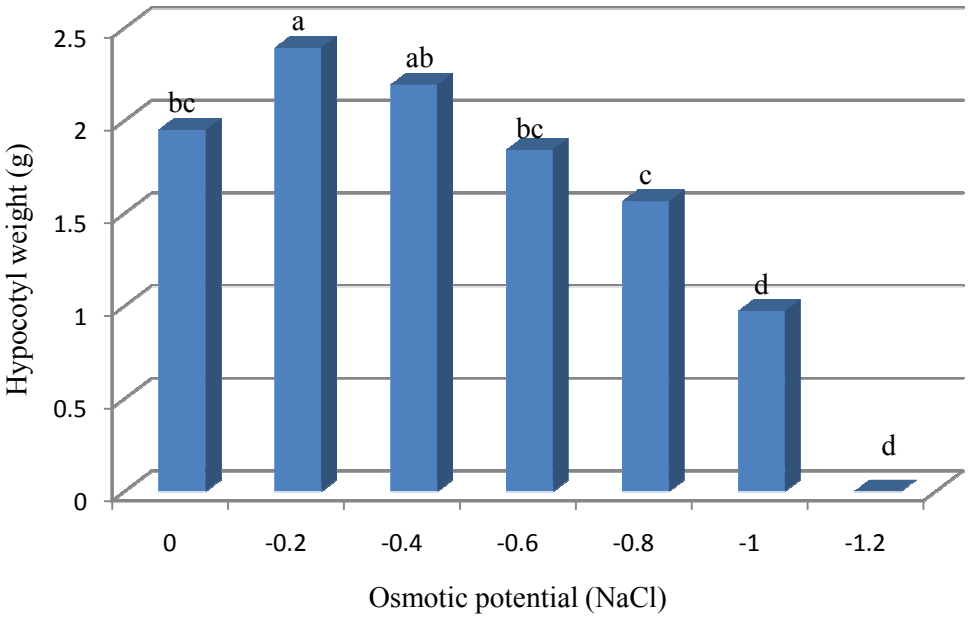

Fig. 2.17. Mean hypocotyle weight in solutions of increasing NaCl concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences *(P* < 0.05) between different osmotic potentials (by Tukey's test).

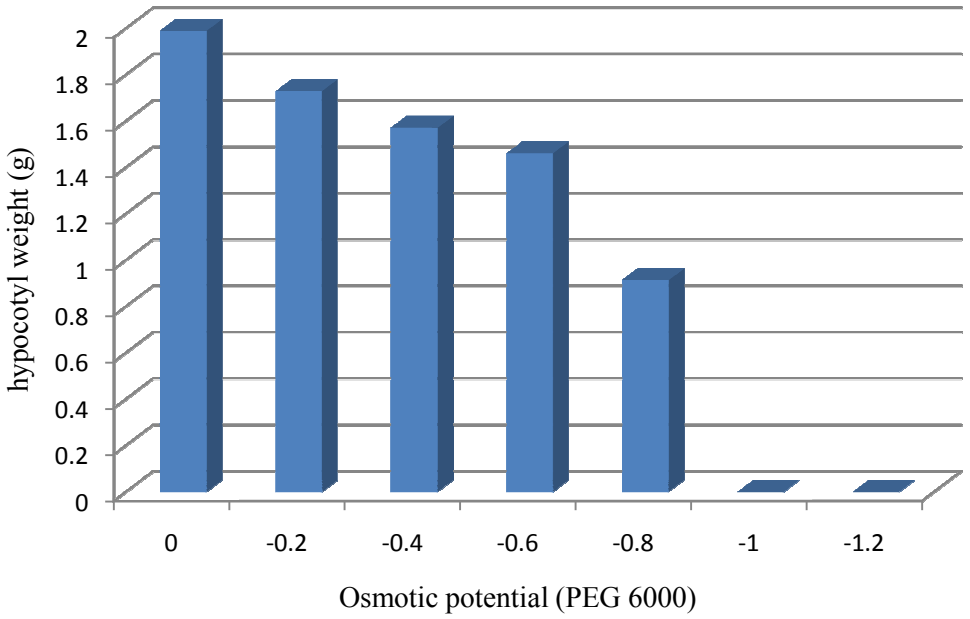

Fig. 2.18. Mean hypocotyle weight in solutions of increasing PEG 6000 concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences *(P* < 0.05) between different osmotic potentials (by Tukey's test).

Root weight of Ensabi decreased by increasing of NaCl solutions. Results showed that although length of the roots was not significantly different between control and moderate of NaCl solutions, the dry weight of roots was significantly lower compared the control. There was no root growth at −1.2 MPa of NaCl (**Fig. 2.19**).

Like those subjected to NaCl solution exposure, dry weight of Ensabi roots was sharply decreased by increasing of PEG 6000 osmotic potentials. although the length of roots that was significantly longer compared with the control in low PEG solutions, the dry weight of roots in the same solutions were significantly lower compare to the control. This results indicated the roots that grew under drought treatment were longer but were very thin and delicate (**Fig. 2.20**).

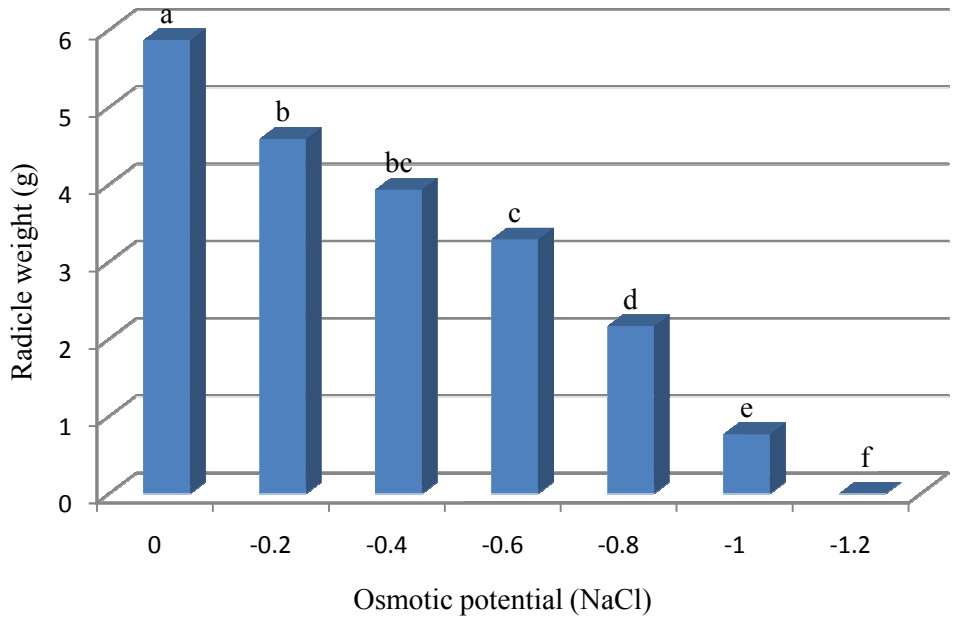

Fig. 2.19. Mean radicle weight in solutions of increasing NaCl concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences *(P* < 0.05) between different osmotic potentials (by Tukey's test).

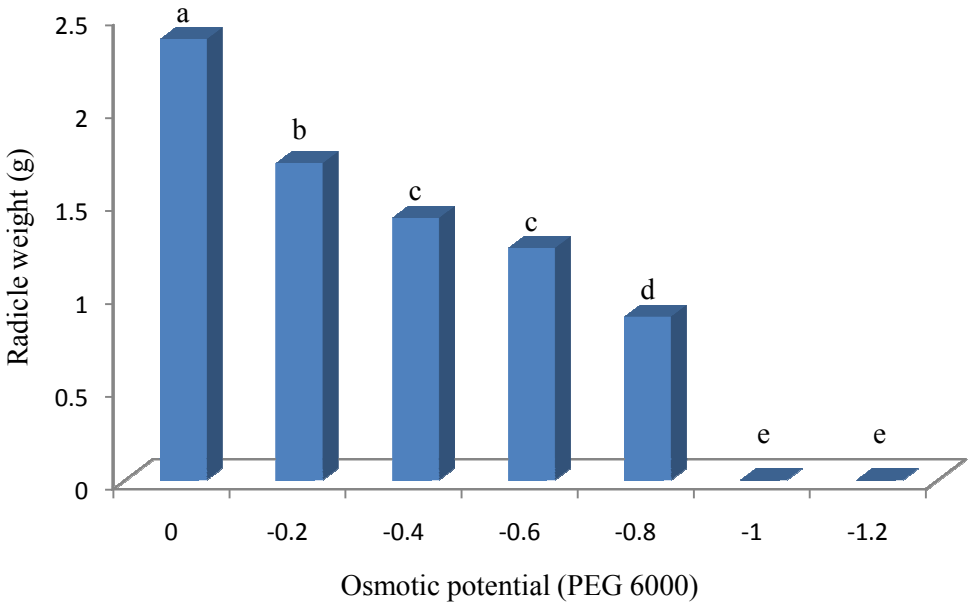

Fig. 2.20. Mean radicle weight in solutions of increasing PEG 6000 concentrations for *Brassica juncea* var. Ensabi. Different letters indicate statistically significant differences *(P* < 0.05) between different osmotic potentials (by Tukey's test).

2.3.3 General growth pattern

The results on the major growth characteristics of *B. juncea* var. Ensabi grown under two light regimes are listed in **Table 2.6** and the regression models (**Tables 2.7** and **2.8**) represent the best-fitted model for different plant characters including plant height, leaf number/plant or leaf number/branch and flowers/branch or number of pods/plant and the days after planting in PEP and FEP conditions. Plant height was elongated with increasing plant age and growth model equations for plant height were the Richard's regression model in PEP and FEP treatments. The regression analysis was generated to analyse plant height in relation to days after planting (**Fig. 2.23**).

Table 2.6. Growth characterestics of *Brassica juncea* var. Ensabi at harvest ubder different light regimes.

Growth parameter	Mean in full light ± SD	Mean in partially light ± SD
1. Plant height (cm)	97.6 ± 2.86	104.7 ± 2.50
2. No. of leaves / plant	89.7 ± 3.46	86.6 ± 3.56
3. No. of nodes / plant	10.6 ± 0.96	10.0 ± 1.24
4. No. of leaves in primary branch No. 1	8.3 ± 0.94	7.5 ± 0.85
5. No. of leaves in primary branch No. 2	17.0 ± 1.15	16.3 ± 2.21
6. No. of leaves in primary branch No. 3	22.2 ± 1.88	22.0 ± 1.76
7. No. of pods / plan	287.1 ± 4.15	234.0 ± 2.54
8. No. of pods in primary branch No. 1	34.0 ± 1.05	29.0 ± 2.04
9. No. of pods in primary branch No. 2	45.0 ± 1.76	32.0 ± 1.56
10. No. of pods in primary branch No. 3	48.0 ± 2.58	36.0 ± 2.76
11. No. of seeds / pod	11.6 ± 1.52	11.1 ± 1.46
12. No. of seeds / plant	3286.0 ± 52.3	2531 ± 41.9
13. No. of primary branches / plant	10.6 ± 1.24	10.1 ± .96
14. No. of secondary branches / plant	16.0 ± 1.69	14.0 ± 1.69
15. Plant stem diameter (mm)	4.2 ± 0.22	3.3 ± 16

Table 2.7. Growth model summaries and parameter estimates of the regression relationships between plant height, leaf numbers/plant and leaf numbers/branch of *Brassica juncea* var. Ensabi as a function of time after transplanting raised in partial sun light.

Dependent variable	Regression type	Regression equation	Standard error	R^2
Number of 2^o Branch /Plant	MMF Model	$y=(0.495+151.398x)/(165.058+ x^{0.711})$	0.156	0.99
Number of Leaves /2^o Branch /Plant	Rational Function	$y=(0.524+0.0.229x)/(1-0.084x+ 0.003x^2)$	0.847	0.98
Number of Leaves /1^o Branch /Plant	Quadratic Fit	$y=0.223+0.486x-0.012x^2$	0.243	0.99
Plant Height (cm)	Richards Model	$y=105.233/[1+\exp(10.123-0.177x)^{1/1.62)}]$	0.979	0.99
Number of Leaves/Plant	Polynomial Fit	$y = -2.161+1.704x-0.118x^2-0.003x^3$	3.67	0.98
Number of 1^o branch /plant	Rational Function	$y= 0.023 + 0.115x/1 - 0.09 x + 0.001x^2$	0.156	0.99
Number of Pods /Plants	MMF Model	$y= -498.45+291.27x^{2.03} / 453.20+ x^{2.03}$	5.077	0.99
Number of flowers /Plant	Sinusoidal Fit	$y= 77.57+78.18 \cos (0.16x-3.54)$	5.690	0.99

Table 2.8. Growth model summaries and parameter estimates of the regression relationships between mean plant height, leaf numbers/plant or leaf numbers/branch of *Brassica juncea* var. Ensabi as a function of time in full sunlight.

Dependent variable	Regression type	Regression equation	Standard error	R^2
Number of 2^o Branch /Plant	Polynomial Fit	$y=0.017+0.305x+.004x^2-7.379x^3$	0.147	0.99
Number of Leaves /2^o Branch /Plant	Polynomial Fit	$y=0.365+0.197x+0.103x^2+0.004x^3$	0.537	0.99
Number of Leaves /1^o Branch /Plant	Rational Function	$y=(0.669+0.068x)/(10.077x+0.002x^2)$	0.347	0.98
Plant Height (cm)	Richards Model	$y=92.68/[1+\exp(9.947-0.178x)^{1/1.256)}]$	2.151	0.99
Number of Leaves/Plant	Polynomial Fit	$y= 1.436+0.552x-0.06x^2+0.002x^3$	5.283	0.98
Number of pods /Plant	Polynomial Fit	$y= 0.0.021-1.73x+ 0.82x^2-0.02x^3$	2.357	0.99
Number of 1^o Branch /Plant	Rational Function	$y= (0.024+0.104x)/(10.02x+0.002x^2)$	0.117	0.99
Number of flowers /Plant	Sinusoidal Fit	$y= 77.57+78.18 \cos (0.16x-3.54)$	5.69	0.99

Significant differences between the growth parameters in two light regimes: (1) full sunlight in an open (at midday, mean photosynthetically active radiation, PAR =1812 µ mole photon m^{-2} s^{-1}) and (2) within the greenhouse (at midday, PAR =384 µ mole photon m^{-2} s^{-1}) (The light readings were taken using a LICOR Radiometer) was observed. Results showed that light intensity significantly affected the number of flowers/plant (**Figs. 2.24** and **2.25**), flowers number one and two primary branches, pods/plant and pods in primary branches number one, two and three. Full light also affected seed weight and stem diameter. In comparison to full sunlight partially sun light affected on plant height (**Fig. 2.21**). Annual crops often exhibit S-shaped growth patterns and the logistic equation has long been used to describe those patterns (Sheehy *et al.* 2004).

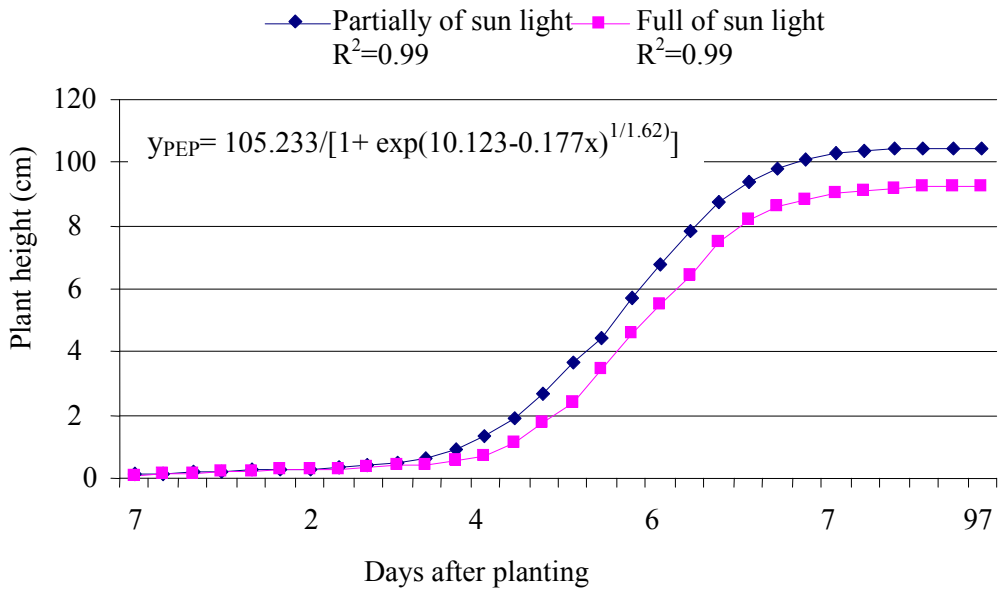

Fig. 2.21. Effect of different light regimes on plant height of *Brassica juncea* var. Ensabi

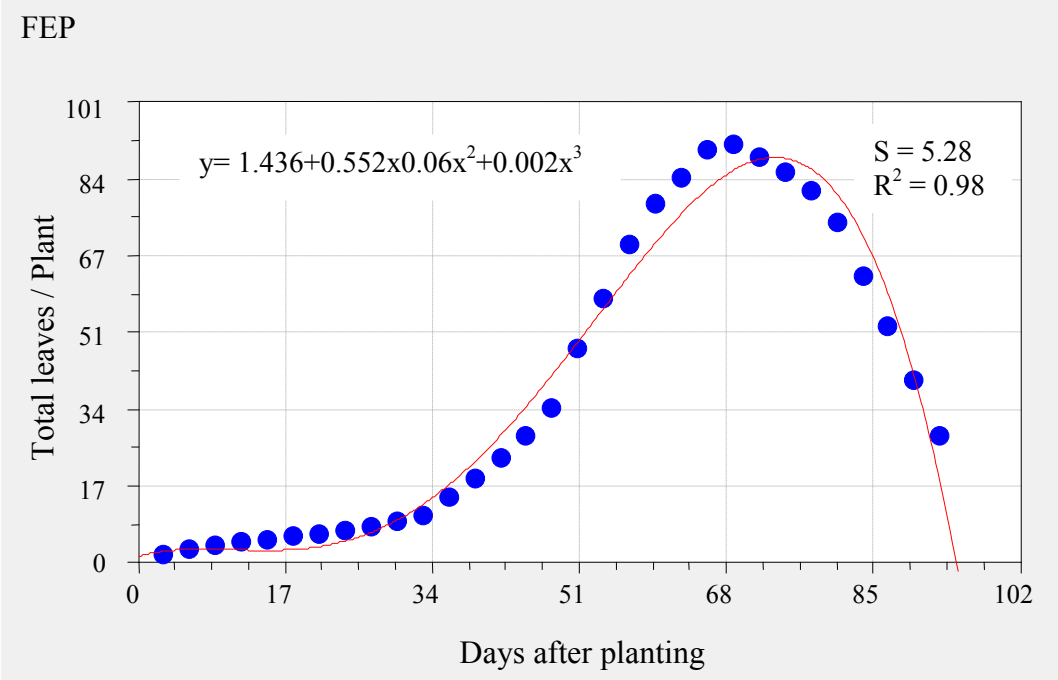

Fig. 2.22. Total number of leaves of *Brassica juncea* var. Ensabi under partially exposed to sunlight (PEP) conditions (384 µmole photon m^{-2} s^{-1}).

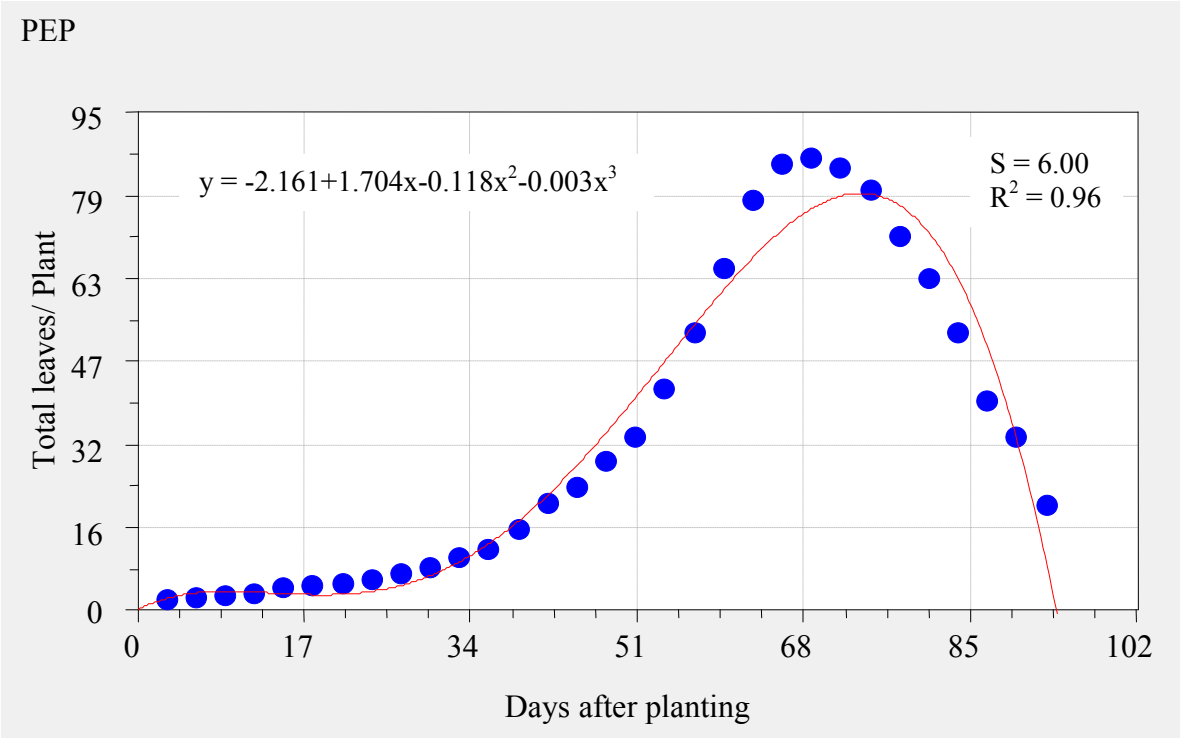

Fig. 2.23. Total number of leaves of *Brassica juncea* var. Ensabi under fully exposed to sunlight (FEP) conditions (1812 µmole photon m^{-2} s^{-1}).

No significant differences were observed in leaves/plant, leaves in primary and secondry branches, number of seeds/pod and number of nodes/plant (**Table 2.6**). By definition "development" is the progress of a plant through the stages of its life cycle and growth is the increase in size of organs and accumulation of dry matter, firstly as sugars and then as structural and storage materials in leaves, stems, roots and grains/pods/fruits (Mendham, Salisbury 1995).

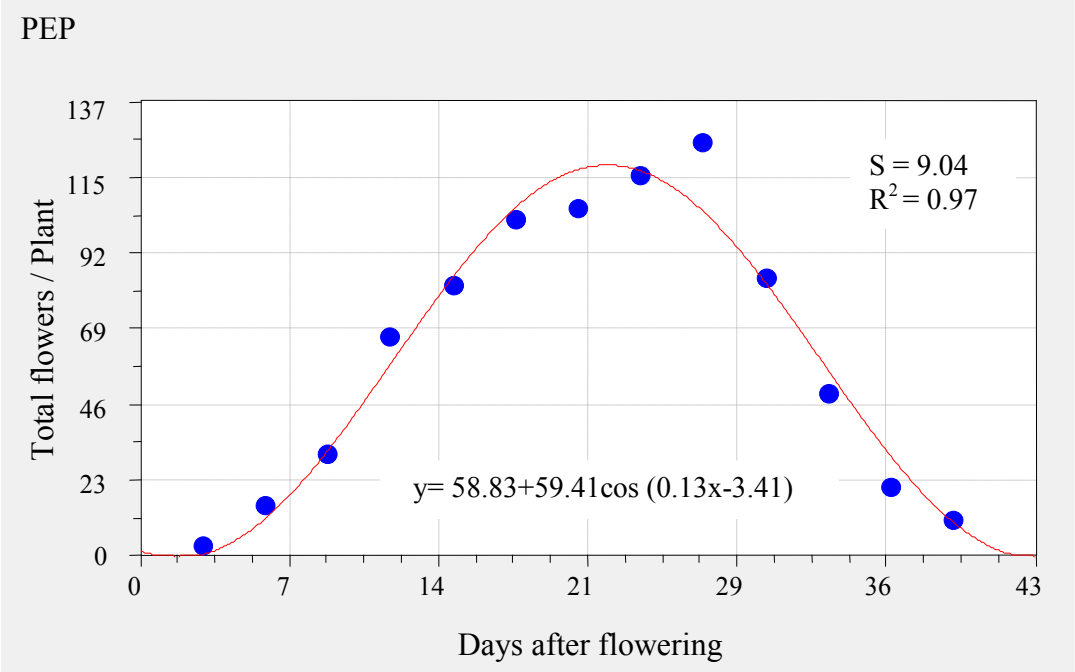

Fig. 2.24. Total number of flowers of *Brassica juncea* var. Ensabi under partially exposed to sunlight (PEP) conditions (384 µmole photon m^{-2} s^{-1}).

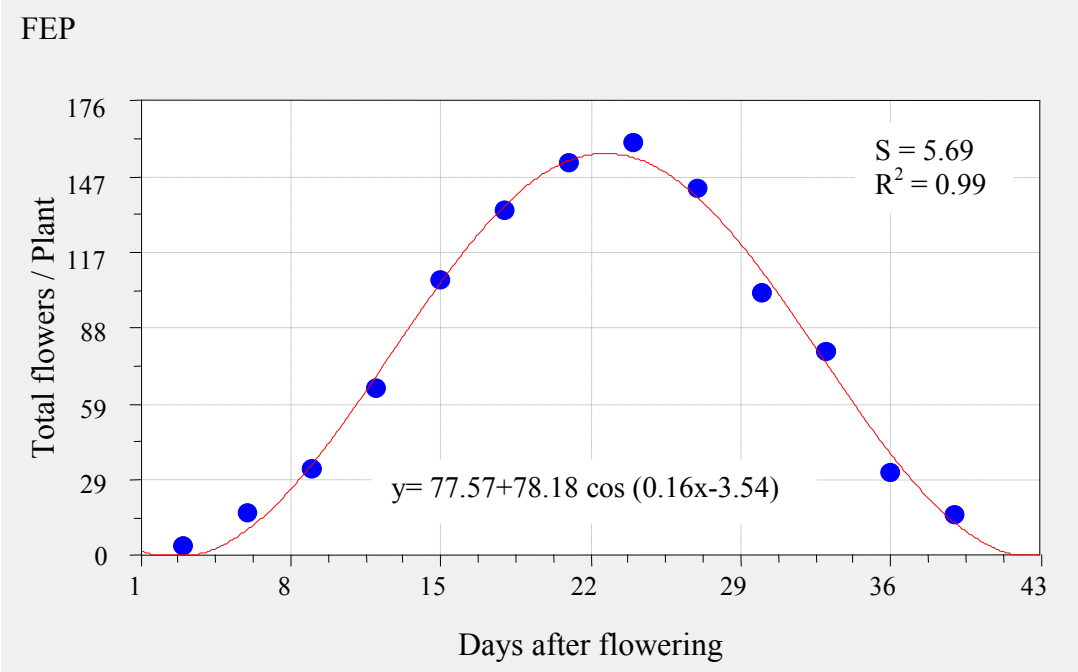

Fig. 2.25. Total number of flowers of *Brassica juncea* var. Ensabi when fully exposed to sunlight (FEP) conditions (1812 µmole photon m^{-2} s^{-1}).

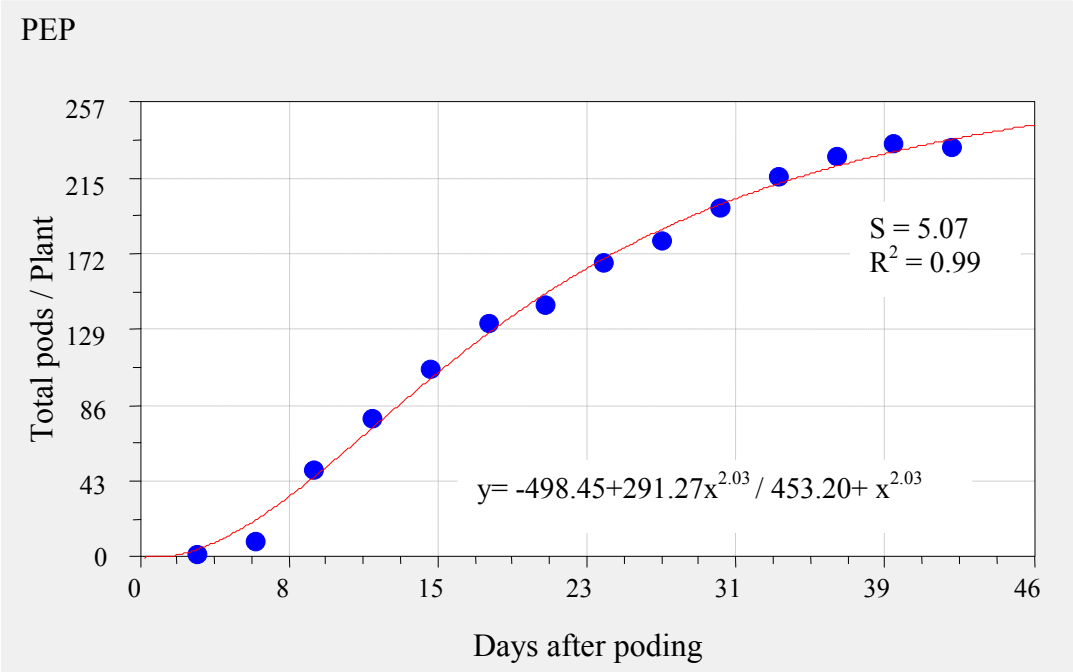

Fig. 2.26. Total number of pods of *Brassica juncea* var. Ensabi when partially exposed to sunlight (PEP) conditions (384 µmole photon m^{-2} s^{-1}).

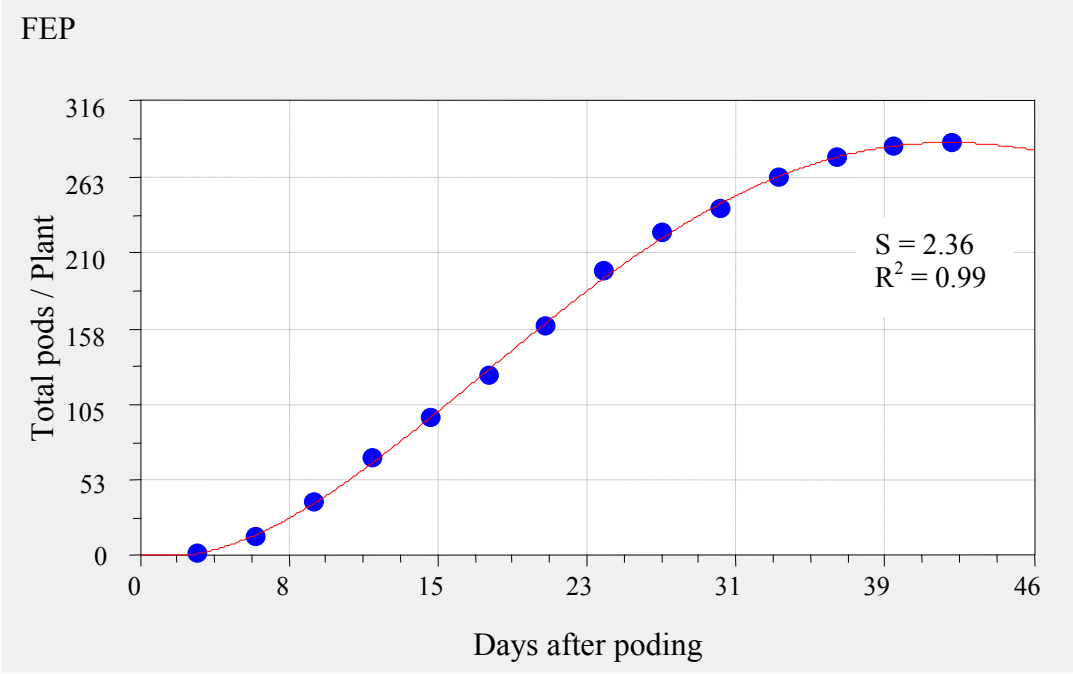

Fig. 2.27. Total number of pods of *Brassica juncea* var. Ensabi when fully exposed to sunlight (FEP) conditions (1812 µmole photon m^{-2} s^{-1}).

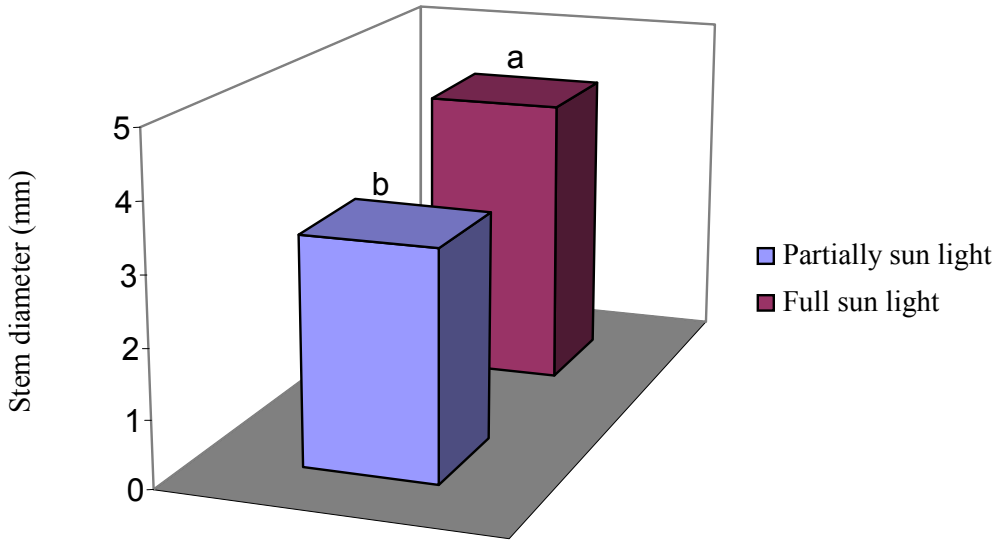

Fig. 2.28. Effect of different light regimes on stem diameter of *Brassica juncea* var. Ensabi.

CHAPTER 3

EFFECT OF NPK FERTILIZER ON THE GROWTH AND SELECTED AGRONOMIC TRAITS OF *BRASSICA JUNCEA* (L.) CZERN. VAR. ENSABI

3.1 INTRODUCTION

The processes of yield formation in plants are highly variable and depend on genetic, environmental and agronomic factors as well as the interactions between them. The yield potential of a crop is a theoretical assessment of the maximum yield that can be generated when high yielding biological material is grown in an optimum physical–chemical environment. In addition, the harvest index (HI), the proportion of seed dry matter allocated to the above-ground biomass, is a major parameter that limits yield (Rathke *et al.* 2006).

Seed yields can be increased by simultaneously increasing the capacity of the sink and the source for seed filling. During crop growth, the supply of nutrients and availability of assimilates for pod set and seed filling are decisive factors that may influence yield. Thus balanced and integrated nutrient supply may not limit yield formation as indicated by higher interception of light and primary photosynthesis, nutrient uptake and biomass partitioning (Mandal and Sinha 2004).

Fertilizers have a pivotal role in plant growth, increasing crop production and specially show significant increase in seed yield, number of grains per pod and oil production in *Brassica* oilseeds. Before the introduction of mineral fertilizers in the nineteenth century, soil fertility was maintained mostly by the recycling of organic materials and crop rotations that included nitrogen-fixing leguminous crops (FAO 2006).

However, under this system of dependence on organic fertilizers in agriculture, periodic famines were endemic. At the beginning of the twentieth century, there was a particular concern about the availability of adequate quantities of nitrogen (N) fertilizers in the Americas. The industrial fixation of atmospheric N resolved this issue. Since the beginning of the 1960s, the large increase in the demand for food, caused by the substantial

increase in the world's population and improved diets, has been met largely by improved agricultural productivity, in which the use of fertilizers played an important role. World fertilizer use has increased almost by five-fold since 1960 (FAO 2006).

The element nitrogen (N) is required in the greatest quantity by most plants to maximize yields, maintain health, and promote growth and also it is the most commonly limiting nutrient in the production of non-N-fixing crops. The oilseed *Brassica* has a high requirement for nitrogen supply, and substantial applications of N-fertilizer are usually needed to obtain maximum yields. Many studies have shown that both growth and yields of oilseed canola are enhanced significantly by high rates of applied nitrogen.

For Indian mustard the sink lies in the pod and seeds and hence under optimum supply of N there is greater translocation of photosynthates from leaves to sink, resulting in robust pod and seeds. To obtain high canola yields, it is important not only to apply the optimum rate of N fertilizer, but also to manage the N application according to the stage of crop development and yield potential (Hocking *et al.* 1997).

Yield response of canola to increasing N rate varies with different environmental variables, including weather, soil type, residual fertility (especially nitrate), soil moisture, and cultivar. Asare and Scarisbrick (1995) showed that high N rates (240 kg N ha^{-1}) gave large effect on yield of rape, and Gan *et al*. (2007) obtained similar results on *B. juncea* varieties. For spring rapeseed, Mandal *et al*. (2006) also reported that seed yield increased with increasing rates of N.

Nitrogen increases yield by influencing a number of growth parameters such as the number of branches and flowers per plant, the number of pods per plant, weight of pods and seeds per plant and by producing more vigorous growth and development such as the total plant weight and the leaf area index (LAI). Wright *et al*. (1988) reported that N prolongs the life of leaves, improves leaf area duration after flowering and increases overall

crop assimilation, thus contributing to increased seed yield. On the other hand, excessive use of N fertilizer can increase lodging with yield and quality reductions.

A significant increase in growth rate of plant and yield has been observed with the application of nitrogen (Hocking 1997). There was a progressive increase in dry matter accumulation, leaf area index (LAI), leaf area duration (LAD) and crop growth rate (CGR) with increasing nitrogen rate from 0-80 kg h^{-1}. However, there was no significant increase after rate of 120 kg ha^{-1}. Qayyum *et al.* (1991) determined the effect of different nitrogen levels (0-150 kg N ha^{-1}) on growth and yield of rapeseed and concluded that nitrogen application up to 120 kg ha^{-1} progressively increased the seed yield, mainly due to increase in number of pods/plant and seeds/pod. However, increase beyond 120 kg N ha^{-1} had adverse effect. Excess nitrogen rate, however, can reduce seed yield and quality appreciably. Bajpai *et al.* (1992) studied the effect of N application on seed yield of mustard. *Brassica juncea* was given 5 N levels (0, 20, 40, 60 or 80 kg N ha^{-1}) along with 30 kg P205 and 20 kg K20 ha^{-1}. Increasing levels of N resulted in higher seed and straw yield. The optimum level of N appeared to be 80 kg ha^{-1}. Kumar and Shaktawat (1992) also reported that when rapeseed cv. T9 was given 0, 30, 60 or 90 kg N ha^{-1} the highest seed yield was obtained at 60 kg N ha^{-1}. The seed oil content decreased with increasing rate of N. Taylor *et al.* (1991) observed that split applications of N were not more effective than application of the total amount of N at seeding.

Nitrogen is a part of the chlorophyll molecule, amino acids, proteins, nucleic acids and pigments. Adequate supply of nitrogen favours the transformation of carbohydrates into proteins and promotes the formation of protoplasm and since protoplasm is highly hydrated, the plant becomes more succulent. Normal metabolic activities can continue only in the presence of optimum level of nitrogen. The addition of nitrogen enhances vegetative

growth and its deficiency leads to stunted growth with small yellow leaves and low production.

N fertilizer increases the biomass production of Indian mustard. Kakati and Kalita (1996) evaluated the response of Indian mustard varieties to different levels of N (0, 25, 50 and 100 kg ha^{-1}). All the yield attributes and seed yield ha^{-1} significantly increased by nitrogen application over control. The number of branches per plant, dry mater, seed, straw and oil yield ha^{-1} was increased significantly with increasing level of N. However, seed oil content was decreased while 1000-seed weight and number of seeds $^{-1}$ were not significantly affected.

According to Kumar *et al*. (1999) phenological development was delayed by increasing nitrogen rates from 0 to 80 kg ha^{-1} while LAI, growth rate and seed yield ha^{-1} of *Brassica juncea* increased linearly with increasing N rates.

The application of nitrogen to canola increased the plant yield. Ahmad (1999) and also Khan *et al.* (2000) demonstrated that application of 80 kg ha^{-1} of nitrogen, ethrel (with ethylene as the active component) affected the parameters favourably with the exception of 1000-seed weight, HI, seed N content and NHI (nitrogen harvest index).

N-fertilizers operate gradually different due to their chemical composition. N is applied as mineral fertilizer rather than organic fertilizer. Depending on the chemical composition of fertilizer, N is available in different from such as nitrate, ammonia, urea, amino acids and acting specifically on seed yield and N-efficiency.

According to Hocking and Mason (1993) although seed yield of *B. juncea* was increased by nitrogen application negligible effect were observed on pod length, 1000-seed weight, number of seeds/pod and seed oil content. Padmani *et al.* (1992) studied the yield response of *B. juncea* to different levels of irrigation and nitrogen rates and observed that although seed yield was increased but seed oil concentration was decreased.

According to Prasad and Ehsanullah (1988) seed yield of *Brassica* was increased by increasing nitrogen level while seed oil content was not affected by nitrogen application. Mohammad et al. (1997) demonstrated that with increasing rate of N, the oil content of *B. juncea* decreased. It was also reported by Singh and Saran (1987) that the application of nitrogen (60 kg N/ha) to *B. campestris* var. 'Toria' although increased the number of pods/plant, 1000-seed weight and seed yield but did not affect the seed oil content. However, plant yield was increased with the increasing rate of nitrogen. Rathore and Monohar (1989) showed that oil content of *B. juncea* significantly increased by application of 90-180 kg N ha^{-1}.

Padmani et *al*. (1992) studied the yield response of *B. juncea* to different levels of irrigation and nitrogen rates and observed that although seed yield was increased but seed oil concentration was decreased. Gupta and Azad (1992) observed the response of raya (*B. juncea*) to combined application of nitrogen and phosphorus and observed an increase in seed yield of plants with increasing rates of nitrogen and phosphorus compared with the control.

Dalai *et al*. (1996) studied the effect of different nitrogen levels on production, efficiency and quality of late sown Indian mustard and reported that increased nitrogen level although increased the seed yield but decreased the seed oil content. Seed oil content showed a negative correlation while seed yield showed a positive correlation with increased nitrogen levels.

Based on results of Narang and Gill (1992), the response of *B. napus* to the application of 0-150 kg N ha^{-1} showed that seed yield was increased from 0.49 to 1.91 t ha^{-1} with increased nitrogen level. Similarly Shukla and Kumar (1994) also reported an increase in seed yield and seed oil content with nitrogen application.

Saleem *et al.* (2000) studied the effect of N, P, K application on the seed yield and oil contents of the three raya (*B. juncea*) cultivars and observed that although growth and yield parameters were increased, none of the fertilizers affected the seed oil contents of all three cultivars of raya (*B. juncea*).

Based on results of Singh *et al.* (1998) application of Nitrogen on *B. nupus* at the rate of 0, 50, 100 and 150 kg N ha^{-1}, increased leaf area index and dry matter accumulation progressively with the application of nitrogen up to 100 kg ha^{-1}. Seed protein content was also increased with increasing nitrogen and up to 100 kg N ha^{-1}.

Nitrogen (N) and phosphorous (P) fertilizers play a vital role in enhancing canola yield. A high rate of N application increases leaf area development, and improves leaf area duration (LAD) after flowering and increases overall crop assimilation, thus contributing to increased seed yield. The beneficial effect of combining N and P fertilizers on P fertilizer use efficiency are well established. Both nitrate and ammonium ions can increase fertilizer P use efficiency. However, the combination of ammonium and P fertilizer is noticeably superior in younger crops and in calcareous soils.

Phosphorus is another important element controlling crop growth and yield in many regions of the world. Phosphorus deficiency reduces plant growth by reducing leaf appearance (Rodriguez *et al.* 1998) and it is a common nutritional problem affecting crop production globally. Billions of hectares worldwide are considered to contain too little P to sustain adequate plant growth.

Phosphorus (P) is a vital crop nutrient for all processes that require energy, synthesis of structural components, and transfer of genetic material. Phosphorus is a key component in adenine triphosphate (ATP), the energy currency of the cell. The energy is used for biosynthesis of metabolic and structural constituents required for plant growth and maintenance. Phosphorus is also an important structural component in plants. It is an

essential component of phospholipids membranes that surround cells, and the organelles within each cell. Coenzymes, nucleotides, phosphoproteins and sugar phosphates all require P for synthesis. Genetic material, such as DNA and RNA contain large quantities of P in the backbone of the molecule (Lehninger *et al.* 1993). Therefore, P is essential for the reproductive function of a plant and thus, P is essential for physiological processes and agronomic crop management.

Phosphorus also plays a role in an array of processes, including energy generation, nucleic acid synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism, and nitrogen (N) fixation (Vance *et al.* 2003).

Phosphorus plays a vital role in several key physiological processes, *viz.* photosynthesis, respiration, energy storage and transfer, cell division and cell enlargement. Phosphorus is an important structural component of many biochemical's *viz.* nucleic acids (DNA, RNA) co-enzymes, nucleotides, phospholipids and sugar phosphate. It stimulates root growth, blooming, fruit setting and seed formation.

Brassica is known to utilize P more efficiently than most of other crops mainly by increased exudation of organic acids in rhizosphere (Vance *et al.* 2003). Rodriguez *et al.* (1998) observed that in a P deficiency, there was a reduction in the rate of leaf expansion and in photosynthetic rate per unit of leaf area. Phosphorus, as a constituent of the cell nucleus, is essential for cell division and development of meristematic tissue. Moreover, P plays a decisive role in carbon assimilate transport and metabolic regulation (starch, sucrose biosynthesis) on a whole-plant scale.

Phosphorus (P) has been found to be the life-limiting element in natural ecosystems because it is often bound in highly insoluble compounds and hence it becomes unavailable for plant uptake or utilization. High soil pH (>7.6) and high quantities of $CaCO_3$ result in 3

precipitation of P, which reduces the soluble P supply. Phosphorus is an essential nutrient and an integral component of several important compounds in plant cells, including the sugar-phosphates involved in respiration, the phospholipids of plant membranes and the nucleotides used in plant energy metabolism and in molecules of DNA and RNA.

Singh *et al.* (2000) conducted a field trial to determine the response of canola and soybean to different levels of P (30, 60 and 90 kg ha^{-1}) and reported that direct application of P, at 60 kg ha^{-1} resulted in higher yield of both crop.

Potassium is considered essential in photosynthesis, sugar translocation, nitrogen metabolism, enzyme activation, stomatal opening, water relation and growth of meristematic tissues, it acts as sieve, root booster, stalk strengthener, protein builder, respiration regulator and retard the diseases, but it is not effective without a co-efficient such as N and P.

Khan *et al.* (2004) studied the response of canola to the application of 0, 25, 50, 75, 100, 125 & 150 $kgha^{-1}$ of potassium fertilization and reported that the highest seed yield (3473 kg ha^{-1}) was obtained with 150 kg ha^{-1} K. Oil content progressively decreased with increase of K level with highest (42.9%) in case of control and lowest (37.4%) with a K level of 150 kg ha^{-1}.

Potassium (K) is an essential macro-element required in large amounts for normal plant growth and development. This attributed to the role of K in plant biochemical pathways. Potassium increases the photosynthetic rates of crop leaves, CO_2 assimilation and facilitates carbon movement. Furthermore, K has an important role in the translocation of photosynthates from sources to sinks.

Potassium application had no effect on number of seeds per pod but it tended to reduce 1000-seed weight of oilseed crops. Hence the yield increase was essentially due to an increase in the number of pods and increased branching of inflorescence. Potassium plays a

major part in the enzyme system that controls the metabolism of photosynthesis and their conversion to oil. It does not usually have a major effect on the seed oil content. Potassium fertilizer had no influence on any aspect of fatty acid composition in any of the crop (Mengel and Kirkby 2004). Similarly, there was no effect on protein of winter rape (Marschner 2007).

El-Aziz (2007) demonstrated that foliar application of NPK fertilizer influenced the vegetative growth of croton plants (plant height, leaves, branches number, root length, stem diameter, leaf area index, fresh and dry weights of leaves, branches and roots).

Malaysia has a total land area of 339,733 km^2, consisting of two geographical regions (West or Peninsular Malaysia and East Malaysia). These regions are separated by the South China Sea. Their climatic and agro-ecological environments are somehow different despite their belonging to the same warm humid tropics classification (FAO 2004).

Malaysia characteristically experiences heavy rainfall (2,540 mm p.a. and above), average daily temperatures of 21-32^oC and a humidity averaging about 85 percent. The seasonal variation in solar radiation is low, resulting in an annual difference in day length of only 2 minutes along the equator and 49 minutes in northern regions. In consequence, there is a year round day length of 12.5 hours (FAO 2004).

About 72 percent of Malaysian soils are Ultisols and Oxisols, which are acidic and highly weathered. Mineral fertilizers account for more than 90 percent of fertilizers used by all types of farming systems in Malaysia. The main fertilizers are urea, ammonium sulphate, calcium ammonium nitrate, phosphate rock, super phosphates, ammonium phosphate, potassium chloride, potassium sulphate and NPK, NP and PK compound fertilizers. Due to the rapid expansion in crop production, especially of plantation crops, there has been a corresponding increase in fertilizer use (Ismail *et al.* 1993; FAO 2004).

There is a paucity of information on the effect of fertilizer on agronomy and ecology of *B. juncea* var. Ensabi in the literature. The primary objective of this study is, to determine effect of N, P, K on some morphological characters, yield and yield components of Ensabi.

3.2 MATERIALS AND METHODS

3.2.1 Study site and experimental design

Seeds of *B. juncea* var. Ensabi, were collected from from Kuching, Sarawak, were sown in plastic pots previously filled with Munchong series (*kaolinitic, isohyperthermic red yellow family*) (Paramanathan 2000) in an insect-proof greenhouse. The plants were subjected to 12 hours of natural sunlight outdoor (mean midday radiation of 1812 µmole photon m^{-2} s^{-1}), and 384 µmole photon m^{-2} s^{-1} inside in insect-proof house, and mean ambient temperatures of 33±2 ^{o}C (day) and 25 ± 2 ^{o}C (night) at Rimba Ilmu, University of Malaya on October 10, 2008.

Before planting the crops, a composite of soil sample was taken from the soils. Samples were thoroughly mixed, filled into plastic bags and brought to the laboratory immediately for drying. The samples were air dried , ground and sieved through 2mm screen (sieve), placed in plastic bags stored at room temperature prior to analysis. The result of analysis is presented in **Table 3.1.**

Table 3.1. Soil fertility status and some physico-chemical characteristics of Munchong series soil, before planting.

	pH	EC (Sm^{-1})	N (%)	P (ppm)	K (ppm)	OM (%)	Sand (%)	Silt (%)	Clay (%)
	5.14	0.96	0.16	21.90	53.00	1.81	61.00	20.00	19.00
Status			Low	Moderate	Very low				

Table 3.2. Treatments and different rates of NPK application on *Brassica juncea* var. Ensabi.

Treatment	Fertilizers	Application rate
T0	Control	
T1	NPK, 50% of farmers experience	75 kg ha^{-1}
T2	NPK, 100% of farmers experience	150 kg ha^{-1}
T3	NPK, 150% of farmers experience	225 kg ha^{-1}
T4	NPK, 50% of farmers experience+ N	75 + 50 kg ha^{-1}
T5	NPK, 50% of farmers experience + N	75 + 100 kg ha^{-1}
T6	NPK, 50% of farmers experience +N	75 + 150 kg ha^{-1}
T7	NPK, 100% of farmers experience + N	150 + 50 kg ha^{-1}
T8	NPK, 100% of farmers experience + N	150 + 100 kg ha^{-1}
T9	NPK, 100% of farmers experience + N	150 + 150 kg ha^{-1}
T10	NPK, 150% of farmers experience + N	225 + 50 kg ha^{-1}
T11	NPK, 150% of farmers experience + N	225 + 100 kg ha^{-1}
T12	NPK, 150% of farmers experience + N	225 + 150 kg ha^{-1}

Treatments consisted of untreated control, applications of different nitrogen, phosphorus and potassium fertilizer NPK (15+15+15) based on farmer`s experience and applications of different N fertilizer. Treatments are shown in **Table 3.2**.

3.2.2 Fertilizer application

Fertilizers were applied in the form of ammonium nitrate (34% N), triple super phosphate and muriate of potash as the source of N, P and K respectively. All the quantity of NPK and half of N fertilizers were applied at the time of planting, while the remaining half of N fertilizer was applied 25 days after planting, because N is commonly the most

limiting plant nutrient, farmers generally apply fertilizers based on N requirements of a crop. All of 'total N' present in a fertilizer is not readily available to a crop when the crop has the highest N demands and also split application of nitrogen fertilizers reduces the leaching and volatilization losses of N. Sidlauskas (2004) reported nitrogen fertilizer applied at sowing, at 4–5 leaf stage and at the start of flowering had very strong and consistent effect on nitrogen concentration in the spring oilseed rape.

Fertilizers were applied in rows 2cm deep and 5 cm away from seeds. The plants were watered once daily, in the morning from above with a fine hose. A seed germination test (as explained in Chapter 2) was conducted to check seed vigour, seed viability and seed germination prior to experimentation.

Measurements of plant growth were done on randomly selected plants. Growth parameters, namely plant height (was measured from the base of the plant to the base of fully open leaf measurement were taken at harvest), number of primary and secondary branches, leaf numbers (was taken as the number of leaflets per plant); and phenological traits (time of flowering, time of starting pod stage and number of pods per plant, seed number/ pod/ mature plant, seed weight/ pod/ plant/mature plant, percent of oil content of seeds (see Chapter 6), stem diameter, above ground fresh biomass (fresh plants after harvesting were separated into leaf, stem and reproductive parts and weighed separately using digital balance) were recorded on different stages of plants life. Plant height and stem diameter were recorded using a metallic measuring tape and a Vernier calliper, respectively.

Plants were harvested at the end of physiological maturity stage and they were dismembered at ground level and separated into stem, leaf, and reproductive parts and oven-dried at 50°C for two

weeks. Finally, these plant components were weighed separately using digital balance and their total dry matter was determind.

3.2.3 Data Analysis

Before analysis, homogeneity of variances at the raw data was checked in each density level, and transformation was used to achieve homogeneity if necessary. The data were subjected to analysis of variance. When significant differences occurred, treatment means were tested for significant differences with Tukey's HSD tests. The data analyses were conducted by using the software package MSTATC and Curve Expert 1.3.

3.3 RESULTS AND DISCUSSION

Results on the effect of NPK fertilizer rates of application on the growth and some selected agronomic traits of *B. juncea* var. Ensabi are presented in **Table 3.3**. Plant height, number of leaves at 40 and 80 DAP and also at maturity stage, number of primary and secondary branches per plant, flowering and pod stages, pod length and stem diameter; were significantly (p<0.05) influenced by different rates of NPK fertilizer (**Table 3.3)**.

Table 3.3. Analysis of variance on morphological characters and agronomic traits of *Brassica juncea* var. Ensabi.

Mean square											
Source	DF	Plant height (cm)	Flowering stage DAP	Pod stage DAP	Maturity DAP	No. of leaves at 40 DAP	No. of leaves at 80 DAP	Leaf No. on maturity	No. of primary branches	No. of secondary branches	Stem diameter (mm)
Replication	3	31.10	12.48	6.63	6.10	8.94	4.84	5.28	0.94	2.53	0.20
Treatments	12	1587.23**	18.05**	13.17*	24.43**	27.13*	191.80*	723.36*	11.03*	72.31*	2.28**
Error	36	7.50	0.41	0.30	0.34	0.84	1.63	4.23	1.03	0.55	0.03
CV %		12.70	11.03	11.79	10.57	13.31	21.99	11.89	18.91	14.62	14.35

* Days After Planting

Table 3.3. ***(cont.).***

Mean square							
Source	DF	Pods / Plant	Pod length (cm)	Seeds / Pod	1000 Seed weight (g)	Seed yield / Plot (g)	Seed oil content (%)
Replication	3	3.46	0.15	7.46	31.10	0.21	0.24
Treatments	12	61.06**	4.39*	78.41**	1587.23**	5.63**	1.00^{ns}
Error	36	0.81	0.24	0.52	7.50	0.35	0.23
CV %		12.80	27.09	12.72	11.56	16.21	11.37

Plant height at maturity was significantly (p<0.01) influenced by various fertilizer combinations over the control. Results indicated that Ensabi plants were higher in NPK and NPK plus extra nitrogen treatments. The maximum rate of fertilizer application (225 kg NPK+ 200 kg N ha-1) produced the highest plant (128.8 cm), while the treatment T9 produced plant height of 121.5 cm and differed significantly. The lowest plant height of 72.3 cm was recorded in control while the treatment T8 and T7 produced 111.3 and 107.3 cm plant height, respectively which were not significantly. T4, T5, T6 graves 90.4, 93.1 and 95 cm plant height, respectively which were significantly lower than the T7 until T12 treatments (**Tables 3.3** and **3.4**). This may be attributed to fertilizer applications and was probably mediated by N-application. Similar results were reported by Kumar *et al*. (1997) in India, Al-Barrak (2006) and El-Aziz (2007).

Plant height is an important growth character directly linked with the productive potential of plant in terms of yield and yield components. An optimum plant height is positively correlated with productivity of plant.

Different rates of NPK significantly affected flowering time of Ensabi. Increasing rate of fertilizer up to T8, plants were slower to reached the flowering time and flowering duration. Control was the first plot that reaches to blossom and T12, T11, T10, T9, T8 were the last treatments reach to flowering stage. Consequently, there were no significant differences between the high rates of NPK on flowering stage of *B. juncea* var. Ensabi (**Tables 3.3** and **3.4**). These findings are in accordance with previous reports of Tewari (1965), Xuewen (1990), Hocking *et al* (1997), Barlog and Grzebisz. (2004) and Gan *et al* (2007).

The time of start pod stage also significantly (p<0.05) influenced by fertilizer applications. By increasing fertilizer rate, the time of start pod stage was significantly delayed. According to results of this experiment, high rate of fertilizer delayed boding of

B. juncea var. Ensabi by five days. Plants at fertilizer application (control) started their pod stages in 69 days after sowing while at high rate of fertilizer application, plants reached this stage at 72 days after planting (**Tables 3.3** and **3.4**).

Table 3.4. Influence of different application rates of fertilizer on some growth parameters at different stages of *Brassica juncea* var. Ensabi.

Treatment	Plant height (cm)	Flowering stage (DAP)**	Pod stage (DAP)**	Maturity (DAP)**
T0	72.25 g*	58.00 g	66.00 f	109.25 h
T1	80.25 f	59.75 f	67.50 e	110.30 gh
T2	81.25 f	61.00 ef	68.00 e	110.50 fgh
T3	84.50 ef	61.25 def	68.25 e	111.00 fg
T4	90.00 de	61.50 cde	68.50 de	111.30 efg
T5	93.00 d	62.00 cde	68.75 de	111.80 def
T6	95.00 d	62.75 bcd	69.75 cd	112.50 cde
T7	107.30 c	63.00 bc	70.25 bc	113.00 cd
T8	111.30 c	63.75 ab	70.50 abc	113.80 c
T9	121.50 b	64.25 ab	71.75 ab	115.50 b
T10	125.80 ab	64.75 a	71.25 ab	115.80 ab
T11	127.00 ab	64.75 a	71.25 a	116.50 ab
T12	128.80 a	65.00 a	71.75 ab	117.00a

* Values followed by similar letters within the same column are not significantly different at $p < 0.05$.

** Days after planting.

Based on **Table 3.4** maturity time also varied statistically. **Table 3.3** referred those fertilizer application rates improved the maturity period of *B. juncea* var. Ensabi. Results indicated that the high rates of NPK+ extra nitrogen significantly ($p<0.05$) delayed the maturity time of Ensabi. Plants with highest rates of fertilizer application took more days (117) t0 compare with the maturity at control level of fertilizer (109.25) days.

Plants receiving 125, 150 and 225 kg NPK plus 50, 100 and 150 kg extra N ha^{-1} fertilizer took more days to mature and significantly influenced the time of maturation as compared to control and other level of fertilizer. This might be due to fertilizer effect on the vegetative growth, which ultimately delayed maturity of the crop. Other treatments took significantly less days to maturity. Results of this research are in line with the results of Ozer (2003) on canola.

The number of leaves in 40 and 80 days after planting and the total leaf number per plant (intact leaves on plant plus dropped leaves) were significantly affected by different fertilizer levels (**Tables 3.3** and **3.5**). Maximum number of leaves in 40 (33) and 80 DAP (75.24) and in maturity stage (124.5)were recorded at highest fertilizer level while minimum number of leaves per plant 24.75, 55 and 81.75 were recorded in control at 40 and 80 DAP and maturity stage respectively (**Fig. 3.1**). These results supported by Gan *et al.* (2007).

Nitrogen is an integral part of chlorophyll which is the primary absorber of light energy for photosynthesis. It is a component of all proteins and strongly increases and support vegetative growth and deep colour, while phosphorus and potassium play a vital role in several key physiological processes *viz.* photosynthesis, respiration, energy storage, cell division and cell enlargement. Therefore the increased number of leaves per plant may be due to balanced fertilization of the Ensabi.

Table 3.5. Selected morphological characteristics of *Brassica juncea* var. Ensabi that were influenced by different NPK sources.

Treatment	No. of leaves at 40 (DAP)**	No. of leaves at 80 (DAP)**	No. of leaves at maturity
T0	24.75 f *	55.00 h	81.75 g
T1	25.75 ef	56.00 gh	90.25 f
T2	25.50 ef	56.50 gh	94.00 ef
T3	26.50 ef	58.25 fg	97.00 e
T4	27.00 ef	59.00 fg	109.50 d
T5	27.50 de	60.75 ef	112.50 cd
T6	27.00 ef	63.25 de	112.50 cd
T7	27.75 cde	66.25 d	115.50 bc
T8	29.50 bcd	70.25 bc	115.50 bc
T9	30.00 bc	69.50 c	116.80 bc
T10	31.50 ab	71.00 bc	119.50 ab
T11	31.50 ab	72.75 ab	122.80 a
T12	33.00 a	74.25 a	124.50 a

* Values followed by similar letters within the same column are not significantly different at $p < 0.05$.

** Days after planting

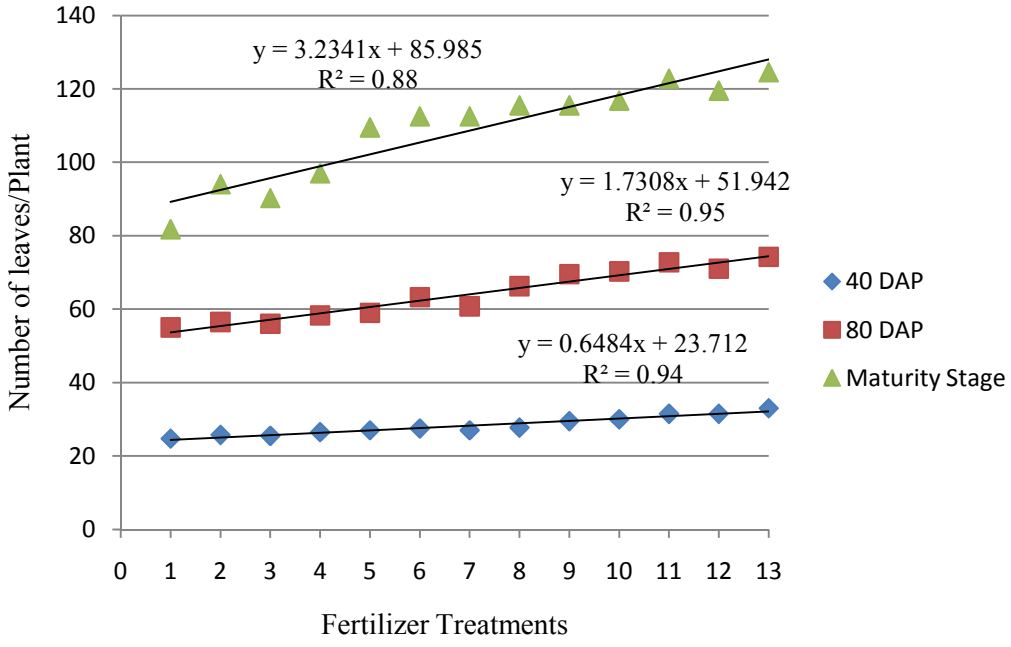

Fig. 3.1. Effect of different fertilizer application rates on number of leaves at 40 and 80 days after planting and on the total leaf number per plant of *Brassica juncea* var. Ensabi.

The results indicated that increasing rates of fertilizer significantly affected number of primary branches of *B. juncea* var. Ensabi. Number of primary branches per plant varied from 8.5 to 13.5, were recorded in non fertilized plot (control) and T9 respectively. Results based on **Table 3.6** revealed that the lowest values of these parameters were recorded in control treatment and the higher numbers of primary branches were recorded when the crop was fertilized with 225kg NPK + 50kg N per hectare. This trend was also observed on number of primary branches/plant although differences not significant between T1, T2, T3, T4, T5 and T7. These can be attributed to increase in absorption and translocation of assimilates and stimulating apical and lateral meristems to grow.

These results were in agreement with established literature (Khan *et al.* 2000; Ozer 2003; Mandal and Sinha 2004; Al-Barrak 2006; Govahi and Saffari 2006; Tunturk and Ciftci 2007; Ahmadi and Bahrani 2009).

Fertilizer application rates significantly affected secondary branches of *B. juncea* var. Ensabi (**Table 3.3**). According to results that presented, application of 225 kg of NPK with additional 150 kg N ha^{-1} (T12) resulted maximum number of primary branches (21.75) per plant **(Table 3.6)**, while it no significant differences were found between this treatment and T11, T10 and T9. T8 gave 19 secondary branches per plant while T7 produced 18 branches/plant but differences were with T9 and followed by (T6) 75 NPK + 150 kg N ha^{-1} have produced (14) number of secondary branches per plant. However untreated control (T0) produced lowest (10) number of secondary branches per plant. Data indicate that, number of secondary branches was significantly increased over control. These results were comperable with the results of different workers (Khan *et al.* 2000; Ozer 2003; Mandal and Sinha 2004; Al-Barrak 2006; Govahi and Saffari 2006; Tunturk and Ciftci 2007; Ahmadi and Bahrani 2009).

Table 3.6. Influence of different NPK levels on number of primary and secondry branches and stem diameter of *Brassica juncea* var. Ensabi.

Treatment	No. of 1° branches	No. of 2° branches	Stem diameter (mm)
T0	8.50 f *	9.50 f	2.97 d
T1	9.00 e f	11.50 e	3.38 cd
T2	9.75 def	12.25 de	3.47 bc
T3	10.25 def	12.25 de	3.59 bc
T4	10.50 cdef	13.50 cd	3.58 bc
T5	11.50 cdef	14.00 cd	3.76 bc
T6	11.25 bcdef	14.25 c	3.92 b
T7	12.00 bcdef	17.50 b	4.64 a
T8	12.25 abcd	18.75 b	4.79 a
T9	13.50 abc	20.75 a	5.04 a
T10	13.00 abc	20.75 a	4.75 a
T11	13.00 ab	21.00 a	5.07 a
T12	13.25 a	21.75 a	5.10 a

* Values followed by similar letters within the same column are not significantly different at $p < 0.05$.

Results indicated that NPK fertilizer significantly ($p<0.01$) influenced stem diameter of *B. juncea* var. Ensabi (**Table 3.3** and **Fig. 3.2**). The plants that were treated with different rates of fertilizer application, produced thicker stems. However differences between high levels of fertilizers were not significant.

Comparison on selected morphological characteristics of *B. juncea* showed that different rates of fertilizer at maturity was the thickest (5.1 mm) and thinner stem diameter (3.38 mm) were recorded in T12 and T1, respectively while non fertilizer treatment plot (control) recorded 2.97 (mm) (**Table 3.6**). Mobasser *et al.* (2008) reported with increase in N fertilizer, the stem diameter was increased.

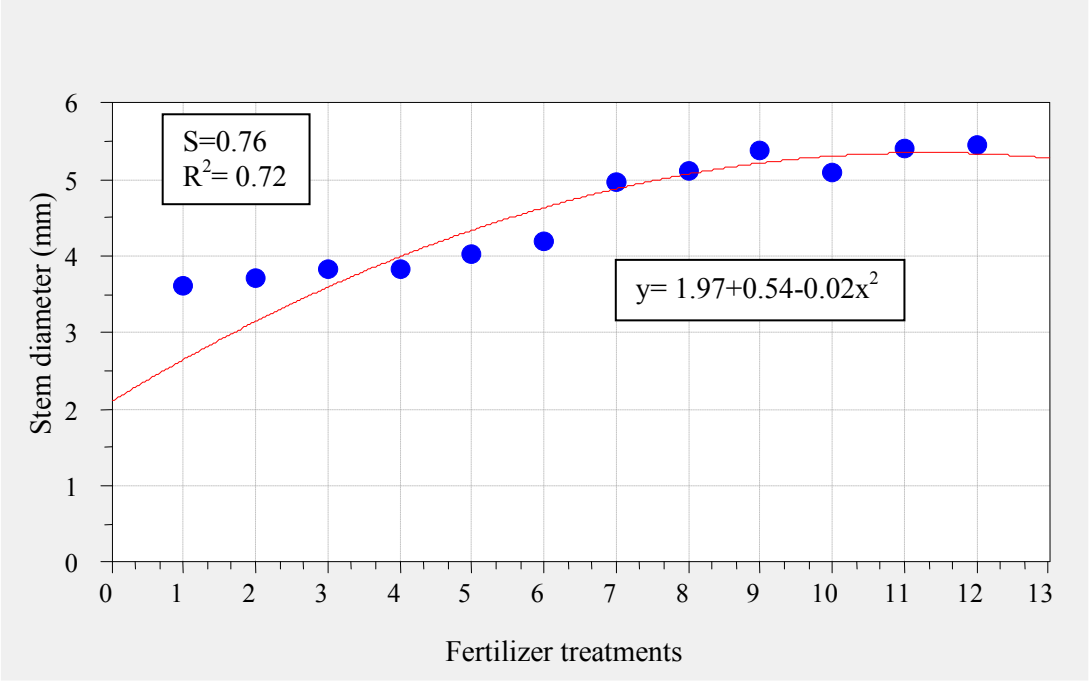

Fig. 3.2. Stem diameter (mm) at maturity of *Brassica juncea* var. Ensabi as a growth parameter that was influenced by different rates of fertilizer.

Number of pods per plant is commonly a major determinant of canola yield and this character ultimately determined by the survival of branches and buds and dependent on the number of flowers produced per plant. Results revealed a significantly differences ($p <$

0.01) in the number of pods per plant were observed amongst the different fertilizer rates (**Table 3.3**). The number of pods per plant increased with increasing rates of fertilizer until T9, but by increasing more fertilizer, the pod per plant was decreased (**Fig. 3.3**).

Number of pods per plant ranged from 107 to 228, when maximum numbers of pods per plant were obtained in T9 where plot treated with 150 kg NPK + 200 kg N per hectare, but there were no significant differences between T9 and T10, when T 10 produced 224.8 pod per plant, while minimum number of pods were recorded 107 where no fertilizer was applied. The numbers of pods recorded were per plant followed by T8 where 150 kg of NPK with additional 100kg N were applied per hectare and numbers of pods recorded were 210.5 per plant and T11 and T12 where 225 kg NPK + 100 kg N and 225 kg NPK + 150 kg N per hectare respectively had given 201.8 pods per plant. These results revealed by increasing fertilizer more than 225 kg NPK with additional 50 kg N, number of pods per plant significantly decreased. All these attributes were higher than with control. This was perhaps due to more number of primary and secondary branches per plant and also may be a balanced ratio of the three macro-nutrients (NPK) influencing positively this parameter.

These results are in line with Schjoerring (1995), hocking *et al*. (1997), Khan *et al*. (2000), Saleem *et al*. (2000), Malik et al (2002), Al-Barrak (2006) and Ahmadi and Bahrani (2009) who reported that increasing the rate of fertilizers increased the pod number over control treatment in Canola.

Pods are the main component contributing to the response of seed yields and seed N accumulation to nitrogen application. The increase in pod number per plant with increasing N fertilizer rate was virtually the only factor responsible for the increased seed yield, as seed number per pod and individual seed weight were comparatively constant over the range of N rates applied.

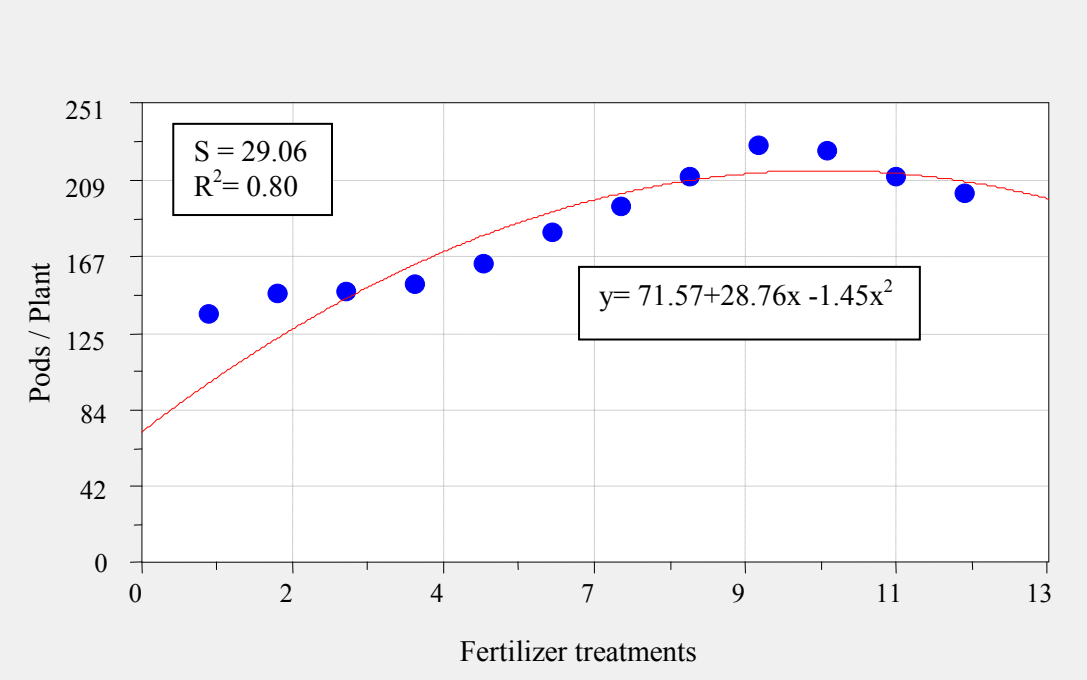

Fig. 3.3. Influence of different NPK levels on the number of pods/plant of *Brassica juncea* var. Ensabi.

There were significant differences ($p<0.05$) in pod length among various fertilizer treatments. Treatment T9 produced the longest pod (4.2 cm) where 150 kg NPK and 150 kg N per hectare were applied. This was followed by treatment T11 and T10 that producing 4.2 and 4.16 cm long pods respectively (**Table 3.7**). Treatments T3, T2 and T1 produced 3. 46, 3.40 and 3.325 cm long pods, respectively, and these were not significantly different with each other. The minimum pod length of 3.01 cm was noted in treatment T0 (control). These findings are similar to ehose of Faramarzi *et al.* (2009). The long pods were obtained from treatments receiving higher fertilizer rates, were probably due to better nutrition status of plant during pod growth period.

Number of seeds per pod is an important yield component contributes materially towards final seed yield of brassica oilseeds. Number of seeds per pod significantly (p<0.05) increased with increasing levels of fertilizer. *B. juncea* var. Ensabi plants in T9, produced highest number of seeds per pod (13.25) as well as longest pods, where 150 kg NPK and 150 kg N per hectare were used.

As evident from the **Table 3.7** the lowest number of seeds per pod (10.25) was found from untreated plot which were given no fertilizer (T0) but differences were not significant between control and T1, T2 and T3 treatments which produced 10.50, 10.25 and 10.75 seeds per pod respectively.

Results showed the treatments that used fertilizer more or less compared to the T9, significantly produced less number of seeds per pod. Treatments T10 and T8 where recorded 13 seed per pod in each treatment and also T11 and T7 were statistically similar where they produced 12 and 12.1 seed per pod, respectively. Likewise for treatments T5 and T12 with 11 seeds per pod each were recorded (**Table 3.7**).

These results are in agreement with the finding of other workers (Khan et *al.* 2000; Saleem *et al.* 2000; Malik *et al.* 2002; Khan *et al.* 2004; Govahi and Saffari 2006; Al-Barrak 2006; Tuncturk and Ciftci 2007; Ahmadi and Bahrani 2009 and Faramarzi *et al.* 2009), who reported that increasing levels of fertilizer application with parallel increase in the number of seeds per pod.

Table 3.7. Influence of different application rates of fertilizer on yield and yield components of *Brassica juncea* var. Ensabi.

Treatment	Pod/Plant	Pod length (cm)	Seed/Pod	1000 Seed Weight (g)	Seed yield/ Plot (g)	Seed oil content (%)
T0	107.00 h*	3.01 h	10.25 c	1.065 f	8.135 d	35.38 a
T1	135.80 g	3.33 g	10.50 c	1.15 e	8.52 cd	35.33 ab
T2	147.30 fg	3.41 g	10.25 c	1.17 e	8.61 bcd	35.20 ab
T3	148.30 fg	3.48 fg	10.75 c	1.21 de	8.87 bcd	35.28 ab
T4	151.80 ef	3.66 ef	11.50 abc	1.25 d	8.93 bcd	35.03 ab
T5	163.00 e	3.72 de	11.00 bc	1.29 cd	9.22 bcd	35.05 ab
T6	180.50 d	3.76 cde	11.75 abc	1.34 c	9.43 bc	34.67 ab
T7	194.30 c	3.89 cd	12.10 abc	1.43 b	9.70 b	34.96 ab
T8	210.50 b	3.92 cd	13.00 ab	1.48ab	10.93a	34.91ab
T9	228.00 a	4.22 a	13.25 a	1.54 a	12.02 a	34.42 ab
T10	224.80 a	4.16 ab	13.00 ab	1.51 ab	11.43 a	34.65 ab
T11	201.80 bc	4.20 ab	12.00 abc	1.50ab	9.69 b	34.49 ab
T12	201.80 bc	3.98 bc	11.00 bc	1.50 ab	8.92 bcd	34.35 ab

* Values followed by similar letters within the same column are not significantly different at p <0.05 (HSD tests).

Yield is the manifestation of various physiological processes occurring in plants and these processes influence the development of plant characters, which are usually modified by imposed management practices. Seed weight has a direct effect on the formation of final seed yield of a crop (Khan *et al.* 2004). A steady and progressive increase in 1000-seed weight of *B. juncea* var. Ensabi was observed with each increment in applied NPK+ N rates up to 150 kg NPK and 150 kg N per hectare (**Table 3.7**). Results indicated that all fertilizer combinations influenced 1000-seed weight significantly (**Table 3.3**). Treatment T9 (150 kg NPK + 150 kg N ha-1) produced the highest 1000-seed weight of 1.53 (g) and differed significantly from all other treatments including control. The lowest 1000-seed weight of 1.07 (g) obtained from the untreated plot (control).

The increase in seed weight at higher nitrogen rates might be primarily due to increase in chlorophyll concentration in levels which led to higher photosynthetic rate and ultimately plenty of photosynthates available during seed development.

Treatments T10, T11, T12 and T8 produced grain showing 1000-seed weight of 1.513, 1.5, 1.5 and 1.48 (g) respectively where 225 kg NPK and 50 kg N, 225 kg NPK and 100 kg N, 225 kg NPK with additional 50 kg N and 150 kg NPK plus 100 kg N respectively per hectare were applied and statistically similar with each other.

The results of the experiments as evident from the **Table 3.6** and **Fig. 3.4,** suggest the role of fertilizer application and specially nitrogen for seed formation in canola. Increased seed weight can be referred to the fact that under nutrient rich soil, more nutrients uptake, increased leaf growth and metabolic activity and faster translocation of metabolites result in heavier seeds.

The treatment T7 where 150 kg NPK was applied and 50 kg N ha^{-1} produced 1000-seed weight 1.433 (g) fallowing T6 where 75kg NPK and 150 kg N per hectare was applied, produced 1000-seed weight 1.34 (g) which differs significantly with each other.

Based on **Table 3.7.**, there was no significant difference observed between T1, T2 and T3 where they produced 1000-seed weight 1.14, 1.16 and 1.21, respectively.

The differences in mean seed weight are generally related to a short period between anthesis and maturity. At this time, supply of assimilates to the pods (seed) plays a crucial role in the development of seed and probably plants with greater supplies of nutrients are at greater advantage than those under low nutrition (Khan *et al*. 2004).

These results were similar to the finding of other workers (Khan *et al.* 2000; Saleem *et al.* 2000; Malik *et al.* 2002; Khan *et al.* 2004; Al-Barrak 2006; Govahi and Saffari 2006; Tuncturk and Ciftci 2007; Ahmadi and Bahrani 2009 and Faramarzi *et al.* 2009).

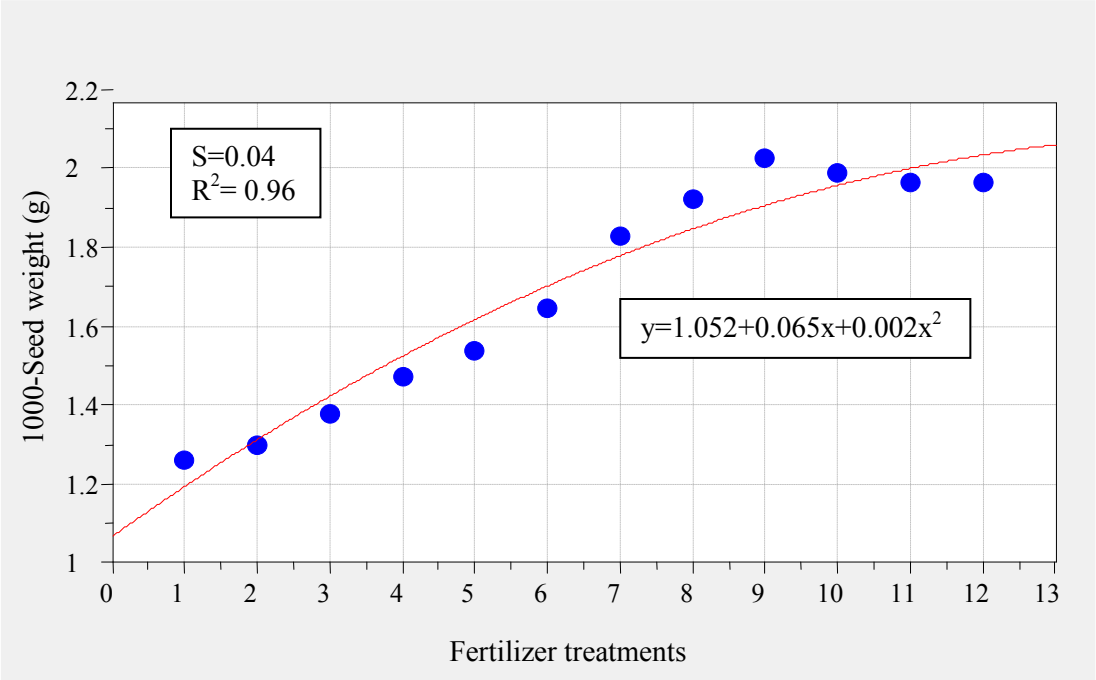

Fig. 3.4. Influence of different NPK levels on the 1000-seed weight of *Brassica juncea* var. Ensabi.

Seed yield per unit area is a function of the combined effect of all the individual yield components, which are influenced differently by the various agronomic practices and environmental factors.

Seed yield of *B. juncea* var. Ensabi affected by NPK (**Table 3.7)**, which indicate that different levels fertilizers had significant effect on the parameter under study. The highest seed yield (12.02 $gplot^{-1}$) was 150 kg NPK and 150 kg N was applied and it was statistically similar to T10 where recorded 11.43 $gplot^{-1}$ seed yield with used 225 kg NPK and 50 kg N ha^{-1} and T8 with 10.93 $gplot^{-1}$ seed yield when 150kg NPK and 100 kg N ha^{-1} was applied. While treatment without any fertilizer application (T0) showed the lowest seed yield (8.135 $gplot^{-1}$) (**Table 3.7**). The highest seed yield was mainly due to increased growth under sufficient macro nutrient status resulting in improvement in components of yield e.g. an increase in number of pods per plant, number of seed per pod and mean seed weight.

The results also showed that there was no significant differences observed between T7 an T11 where they produced 9.695 and 9.686 when fertilizer applications 150 kg NPK and 50 kg N ha^{-1} and 225 kg NPK and 100 kg N ha^{-1} were applied respectively.

The results in **Table 3.7** indicated that the highest rate of N fertilizer application combined with high rates on NPK fertilizer, depressed the seed yield of *B. juncea* var. Ensabi. Treatment No. 12 by 8.92 g $plot^{-1}$ seed yield, significantly produced less seed yield to compare the T9 and T10 and T8. The reason for the low production in seed yield at high rate of N application (T12), is due to the decreased number of pods per plant, number of seed per pod and 1000-seed weight, which accordingly decreased seed yield.

The results of this experiment showed an adequate application of N fertilizer enables the crop to produce rapid leaf growth which may positively contribute in seed filling. This is reflected in efficient partitioning of assimilate into economic yield. The trend in this part of the study is supported by previous studies of (Kumar *et al*. 1997; Khan *et al.* 2000; Saleem *et al.* 2000; Khan *et al.* 2004; Al-Barrak 2006; Govahi and Saffari 2006; Ahmadi and Bahrani 2009 and Faramarzi *et al.* 2009).

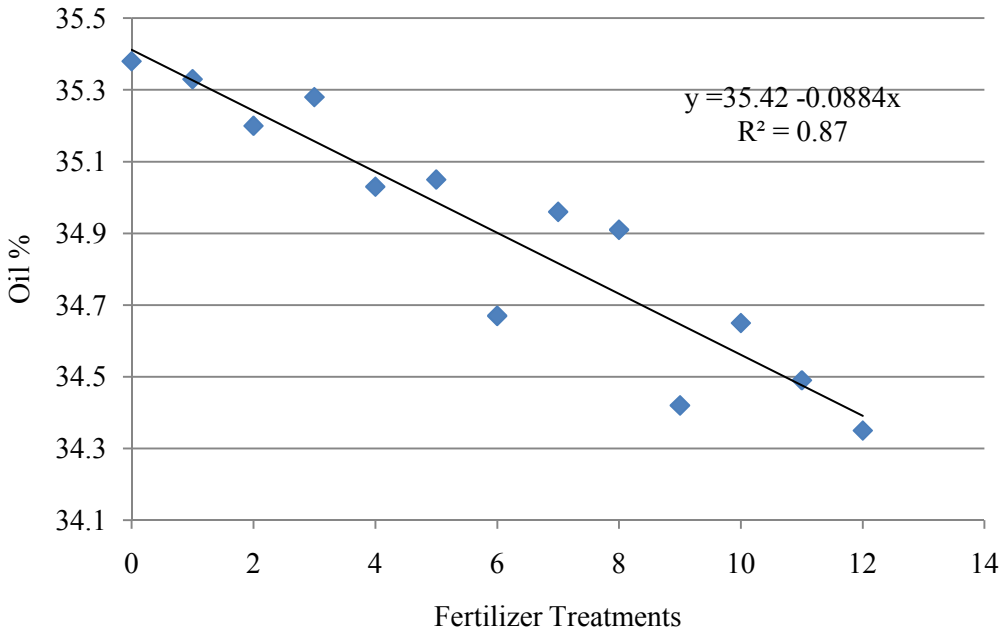

Fig. 3.5. Influence of different NPK levels on the seed oil content of *Brassica juncea* var. Ensabi.

Fertilizer did not significantly affect the oil content of seeds of *B. juncea* var. Ensabi (**Table 3.3**). Data in **Table 3.7** exhibit those effects of different levels of fertilizer applications on the parameter under treatment.

The maximum oil content (35.38 %) was found in T0, (Control) where no fertilizer was applied. The Tl2 treatment (225 kg NPK and 150 kg N ha^{-1}) gave the lowest oil content (34.35%). However the difference in oil contents of other treatments were statistically similar as well as among of oil content of T12 (**Fig. 3.5**).

Although the difference in oil content in the seeds and pods was not significant, there was a decreasing trend oil concentration with fertilizer especially nitrogen. This might be due to relatively high seed yield for the high nitrogen treatments thus causing a dilution effect or could be attributed to the disturbance of carbohydrates translocation mechanism.

The research on oilseed crops has shown that the crops responded differently to fertilizer application especially in seed oil content. These results were in agreement with those of other workers (Hocking *et al*. 1997; Saleem 2000; Leilah and Al-Khateeb 2003; Al Barrak 2006) and in contrast with the results of Malik *et al.* (2002), Khan *et al.* (2004) and Ahmadi and Bahrani (2009).

CHAPTER 4

POPULATION STRUCTURE AND ITS INFLUENCE ON SELF-THINNING OF *BRASSICA JUNCEA* VAR. ENSABI

4.1 INTRODUCTION

Competition is "an interaction between individuals, brought about by a shared requirement for a resource in limited supply, leading to a reduction in the survivorship, growth and/or reproduction of at least some of the competing individuals concerned" (Begon *et al.* 1996; Wilson *et al.* 2007). It can also be defined as "competition is defined as 'the tendency of neighbouring plants to utilize the same quantum of light, ion of a mineral nutrient, molecule of water, or volume of space" (Grime 2001).

Intra-specific competition is a particular form of competition in which members of the same species compete for the same resources in an ecosystem (e.g., food, water, light, nutrients and space). This can be compared with inter-specific competition in which different species compete (Solomon *et al.* 2002). Under competition, the form or size of a plant will become modified without leading to death of the plant. These modifications are known as plastic responses to competition (Harper 1977).

The actual mechanisms of intra-specific competition in any real-live situation involve: a) Attainment of resources by individuals, b) Disturbance in resource acquisition by other members of the population, c) The limited availability or supply of resources.

The early study of intra-specific competition in plants was stimulated by the needs of foresters and agronomists to determine the optimum spacing of plants for maximizing yields.

When a plant grows, consumes more resources and as the individual plant increasing in size and begin to interfere with another's growth by competing for the same resources, so that low light, space, low nutrients and other resources are available for neighbouring plants. Eventually, as all plants in a community grow, increase the size and consume more resources, some plants will not have enough space and resources to survive, and will die. In the other words in the first stages of

growing, the plants grow without interacting, the number of plants and thus the number of plants per unit of area, does not change. In the second stages, the plants begin to interact and many of the plants die off in competition for resources. Finally, the community reaches the self-thinning line, which has a slope of -3/2. The state of the community moves along this line until all mature plants die. This mortality is defined as density-dependent and increases when the density of the community increases. Such density-dependent mortality occurs in monocultures of many plant species, both woody and herbaceous (Harper 1977). When crowding is sufficient to cause death, paths run from the high density-low biomass regime towards lower density-higher biomass (Smart 1986; van der Werf *et al.* 1995) more over mean plant size is related to the reciprocal of population density (Kikuzawa 1999).

A plant thinned by plants of a different species is alien-thinned; a plant thinned by plants of its own species is self-thinned (Harper, 1977) in other words under high densities the stresses of intra-specific competition result in death of the less competitive members of the population. This density-dependent mortality has been termed "self-thinning" (Yoda *et al.* 1963, Harper 1977). A study conducted by Yoda *et al.* (1963) investigated that by sacrificing some individual plants, an overcrowded population of plants is able to produce sufficient seeds for the next generation. This is an important self-regulatory mechanism and has become known as "self-thinning." The self-thinning line is a very robust pattern, which can be obtained in modeling studies by a variety of different mechanistic assumptions (Wieganda *et al.* 2008).

The study of intra-specific competition is concerned with the response of the individual plant within the population and not the response of the total plant population. Competition between individual plants may lead to the process of thinning. Thinning processes may occur in all crowded plants and animal populations, pure or mixed and this may play important roles in the demography of populations and community structure (Quinones *et al.*, 2003).

Much information on the density responses of plants has been obtained from studies on annual plants and from trees (Harper, 1977). Donald (1951) demonstrated that plant yield increased in proportion to planting density at low densities but approached an asymptote at higher planting densities. In self-thinning of plant populations, size inequality decreases as the result of the predominant mortality of the smallest size class (Weiner *et al.* 2001; Rivera and Scrosati 2008)

By understanding on the factors limiting the growth of the individuals within the population, the response of the population as a whole can be deduced. This finding, also known as the "law of constant final yield" (Kira *et al.* 1953), has been shown to hold true for a large number of species. A widely-accepted generalization about these paths is the self-thinning rule, or Power Law of Self-thinning (Yoda *et al.*, 1963, White and Harper, 1970, Lonsdale and Watkinson 1982, Simard and Zimonick, 2005), otherwise known as Self thinning Law or the -3/2 Power Law or Yoda Law and accepted widely ((Yoda *et al.*, 1963, Wieganda *et al.*, 2008, Faravani and Baki, 2009).

Yoda *et al.* (1963) reported that the self-thinning line relates the (log) mean density of plants to the (log) mean plant size with a slope of −3/2, depending on whether plant size is measured in terms of plant volume and plant biomass. This rule supported by many data sets of mean plant size and plant density (Yoda *et al.* 1963; White and Harper 1970; Westoby and Howell 1986; Enquist and Niklas 2001; Wieganda *et al.* 2008). Self-thinning is preceded by increased variability in the size of individuals, with large plants suppressing smaller ones (Harper, 1977). A reduced variability of plant size might therefore reduce or delay the onset of self-thinning (van der Werf *et al.* 1995).

The self-thinning rule describes a relationship between size and density in even-aged plant populations that are crowded but actively growing and relates plant mass to plant density in crowded conditions by the -3/2 power law or Yoda's law (Li *et al.* 2000; Rivera and Scrosati 2008).

Under high densities, the stresses of intraspecific competition result in death of the less competitive members of the population. In nature, however, seeds and seedlings commonly occur at very high densities (Smart, 1986). It was first proposed by Yoda *et al*. (1963) to take the form:

$$w = kd^{\beta}$$

(1) where w is the mean biomass per plant of the surviving plants, d is the number of surviving plants; k and β are the self-thinning coefficient and the power exponent, respectively.

The exponent β has been claimed to take the value −3/2 approximately regardless of species, age or site conditions and k varies with species and growth conditions and it is constant. The relationship of Equation 1 plotted on logarithmic coordinates shows a straight line of slope of -3/2 and this line is called the self-thinning line. This relationship has been confirmed for a large number of species (Yoda *et al.* 1963; Harper 1977; Smart 1986; Rivera and Scrosati 2008). Power-laws are well known to biologists in the form of bivariate relationships of power-law type, otherwise known as scaling relationships (Brown *et al.* 2004; Marquet *et al.* 2005) by which molecular, physiological, and ecological and life history attributes, which in turn relate to some attributes of organisms raised to a power as in equation 1.

In order to simplify curve fitting, data are generally log-transformed to achieve linearity, and analyzed by linear regression techniques. The equivalent Equation 1 is Equation 2:

$$\text{Log w} = \text{Log } K - 3/2 \text{ Log } d \qquad (2)$$

where w is mean weight per surviving plant and k is a constant.

An equivalent relationship also exists between the total stand biomass per unit area, B and plant density, d,

$$B = kd^{-\alpha} \qquad (3)$$

where $\alpha = \beta + 1$ and is approximately -1/2 (Yoda *et al.* 1963; Westoby and Howell 1986; Bi *et al.* 2000).

While the -3/2 power law has generally been used to describe the density-dependent mortality occurring as a result of intraspecific competition (White and Harper, 1970, Harper, 1977), it has also been demonstrated to represent the upper boundary of biomass attainable under a given density of plants (Gorham, 1979).

White and Harper (1970) also indicated that a wide range of species, including herbs, shrubs, and trees, self-thin along a narrow band of -3/2 slope. The log K intercepts for these diverse species range from 3.5 to 4.3, which is quite a narrow range in relation to the wide ranges in biomass and density. These observations indicate a general similarity in the biomass-density relationship among all species examined and suggest that biomass packing is similar in spite of wide variations in plant geometry.

Since the initial acceptance of the rule, the slope of Equation 3 has been found to be much more variable than a constant of -0.5 as the rule states. The debate on the rule holds a great deal of interest in studies on plant population dynamics. The evidence for this rule is examined and then re-analyzed to evaluate objectively in support for the rule. Mathematical models can be constructed to produce testable predictions about causal factors (Bi *et al.*, 2000). The self-thinning rule for even-aged plant populations has been reviewed by Kohyama (1992). Irrespective of the plant types, the rule widely accepted but poorly understood generalization predicts that, through time, growth and mortality in a crowded population trace a straight thinning line of slope -3/2 in a log-log plot of average plant weight versus plant density (Yoda *et al* 1963; Pickard 1983; Rivera and Scrosati 2008).

Many ecological scientists have devoted themselves to research on the self-thinning law for many years, and none of this research has disproven the -3/2 or -4/3 self-thinning law (White and Harper 1970; Harper 1977; Zhang *et al.* 2005). However, the rule certainly does not depend on all

plants in the stand being of the same size at any given time. The hypothesis of the self-thinning line -3/2 (different slope or intercept) may be influenced, hence changed by the fertility level and in high-density populations.

Fertility level may affect on the slope of the thinning lines for both shoot and root biomass from -0.50 to 0.94 and shoot biomass is more sensitive to fertilizer and density levels than root biomass (Morris 1999).

In the same vein, the rule has been hotly debated whether a true self-thinning law exists in nearly pure stands of post-fire chaparral; and if so, is it a -3/2 or -4/3 law (Weller 1987; Guo and Rundel 1998; Bi 2004; McCarthy and Weetman 2007). Lonsdale and Watkinson (1982) showed the -3/2 power law is a characteristic of shoots but not of whole plants of *Lolium perenne*. Increased values in shoot/root ratio prevail in populations as they developed and established so that the thinning line for shoot plus root weight per plant was shallower than the thinning line for shoot weight per plant. Shoot/root ratios were generally higher in shaded than in non-shaded populations (Wright and Fidelibus 2004). Self-thinning rule defines a straight upper boundary line on log-log scales for all possible combinations of mean individual biomass and density in plant populations. More experimental field studies should be carried out to identify the more accurate self-thinning exponent.

Self-thinning rule is widely accepted as an empirical generalization and quantitative rule that applies across the plant kingdom. However, the evidence supporting it has recently come under critical scrutiny. The intrigues on theoretical and empirical bases for the density–mass boundary are still unsettled (Morris 1999; Li *et al.* 2000).

In this study, we wanted to determine if the evidence could support acceptance of self-thinning rule as a quantitative biological law in *B. juncea* var. Ensabi. By using the slopes and intercepts of size-density relationships as variables, the slopes can be explained by simple geometric

arguments. Here, we hypothesized that the sensitivity of plant-to-plant density may affect the rate of self-thinning in the plant community through plant strategy for resource capture and utilization.

4.2 MATERIALS AND METHODS

4.2. 1 Plant establishment

Seeds of *B. juncea* var. Ensabi were collected from from Kuching, Sarawak, Malaysia. A synthetic population of *B. juncea* var. Ensabi was established by sowing seeds directly into 26 cm depth x 14 cm diameter black plastic pots, filled with garden soil of Malacca series (Mca) (Paramananthan, 2000) in an insect-proof greenhouse with 12 hours of natural sunlight (mean midday radiation of 1812 and 384 μmole photon m-2 s-1 outdoor and inside an insect-proof house, respectively). The mean ambient temperatures were 33±2^{o}C (day) and 25±2^{o}C (night) at Rimba Ilmu, University of Malaya, Kuala Lumpur. The plants were watered once daily. A seed germination test (as explained in Chapter 2) was conducted to check seed vigour, seed viability and seed germination prior to experimentation. Five sowing densities, viz. 77, 154, 308, 616 and 1232 seeds were sown in each plastic pot (with area of 0.0154/m2) in ten replications (**Table 4.1**).

Table 4.1. Sowing densities (seeds m^{-2}), Number of seeds per pot sown within an area of 0.0154 m^2 and seedling density (m^{-2}) in *Brassica juncea* var. Ensabi.

Sowing density treatments	Number of sown seeds / pot	Initial number of sown seeds (m^{-2})	(%) of emerged seedlings/pot (10 DAP)	Seedlings / m^2 (10 DAP)*
1	77	5000	63.69	3184.71
2	154	10000	60.16	6015.57
3	308	20000	39.46	7891.01
4	616	40000	28.31	11323.43
5	1232	80000	17.69	14154.28

* DAP: days after planting

Seeds were scattered as evenly as possible on the substrate surface. Mixed soil was irrigated carefully to avoid water stress or seed moving as seeds were sowed at 1-2 cm depth on soil surface.

4.2.2 Recording of density and individual weight of plants of *B. juncea* var. Ensabi.

Samples were taken for the centre of each pot by a circular quadrate (using PVC tube, internal diameter 3.0 cm) at 10^{th} (t1), 20^{th} (t2), 30^{th} (t3), 40^{th} (t4), 50^{th} (t5), 60^{th} (t6), 70^{th} (t7), 80^{th} (t8), 90^{th} (t9) and 100^{th} (t10) days after planting (DAP). The small quadrate (1.8 cm^2) was used in the populations of *B. juncea* var. Ensabi with high density, and the large quadrate (7.1 cm^2) in the populations with low density. The total number of plants in the quadrate was recorded as number per m^2. All fresh plants were killed by liquid N_2 to stop their metabolism before being dried for 48 h at 70° C. At each harvest, several pots were sampled at random. The biomass (dry weight) of root, stem, and leaf was measured after drying. On the 125^{th} day after the *B. juncea* var. Ensabi had been

planted in pots, the remaining plants were harvested and separated into stem, leaf and root. The dry weights of plant parts were obtained after oven-dried.

Nearly all seedlings from a given sowing emerged within 4-5 days after sowing. The emerged seedlings were counted 10 days after planting (DAP). They were considered as the initial densities in this study. Densities at this first observation were checked to see if they were less than the numbers of seeds we had expected to germinate, based on the weighed seed lots for each pot and on estimates of seeds/pot (**Table 4.1**). Therefore, failures to germinate and mortality before emergence were negligible. The mean seed germination percentage at 25^{o} C was 95.48% in the laboratory tests (see Chapter 2).

4.2.3 Data Analysis

Before analysis, homogeneity of variances in the raw data was checked in each density level, and transformation was used to achieve homogeneity if necessary; the power of self-thinning was calculated with formula (2):

$$(\log w = \log K + \beta \log d),$$

where w and d are the average weight and the real density, respectively, of surviving individuals (called 1, 2, 3, 4 and 5 with respect to initial plant densities) (see **Table 4.1**) and K and β were the constant and the power of self-thinning, respectively.

The data were subjected to analysis of variance. When significant differences occurred, treatment means were tested for significant differences with Tukey's HSD tests. The data analyses were conducted by using the software package SPSS and Curve Expert 1.

4.3 RESULTS AND DISCUSSION

Clear differences between densities of established plants of *B. juncea* var. Ensabi and initial sowing were found. Results showed the mean density of established plants during the first measurement after emerging compared with the initial sowing density was extremely lower at all densities employed and ranged from 64% of initial density at low density to 18% at the highest density treatment (**Table 4.1** and **Fig. 4.1**). Substantial declines in density were recorded with time by the first harvest and following harvests at the five density treatments over the study period of 125 days after sowing; the declines were density-dependent.

In the present study the self-thinning effect and results of negative density-dependent seed germination, population numbers decreased from the initial sowing densities. According to **Fig. 4.1**, the relationships at any given time between actual population density and initial population density of *B. juncea* var. Ensabi. In the time course of self-thinning, plant populations cannot be denser than an asymptote of population density whose level becomes lower as time progresses (Hagihara 1999).

Results showed that, as density increases and competition becomes more intense the overall decline in plant numbers was greatest at high sowing density (**Table 4.1**). There was a strong negative linear relation between (%) seed germination (y) and sowing density (x), i.e. y= 60.46-0.0006 x with $R^2 = 0.91$, $p < 0.05$ for inhibition of germination by increasing sowing density (**Fig. 4.2**). This is supported by the results of Lonsdale and Watkinson (1982) and Miller *et al.* (1994) on this negative relationship in other plants.

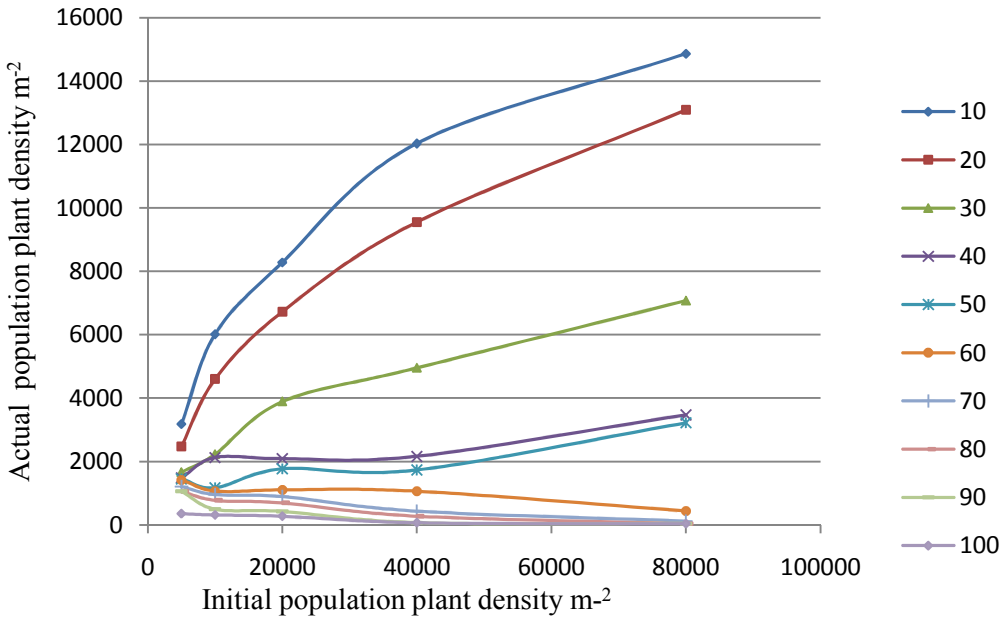

Fig. 4.1. Relationship between actual population (survivors) density and initial population density at each of the given time (10, 20,…, 100 days) in *Brassica juncea* var. Ensabi stands.

The results of this research showed at the highest initial sowing density of 80000 seeds/m^2, only 14154 seeds (17.7% of seeds) emerged, in comparison with the lowest sowing density at 3184 seeds/m^2 (64% of seeds). There are, however, a number of reports in most such experiments, where overall density remained constant while seeds were aggregated in clumps of varying sizes. Seed density and solution volume significantly affect seed germination and early growth of seedlings. The CO_2 produced by the roots might be acting as an inhibitor, and competition for resources between seeds and seedlings may affected seedling densities wherever viable seeds lie close to each other (Inouye, 1980, Lonsdale and Watkinson, 1982, Sinkkonen, 2005).

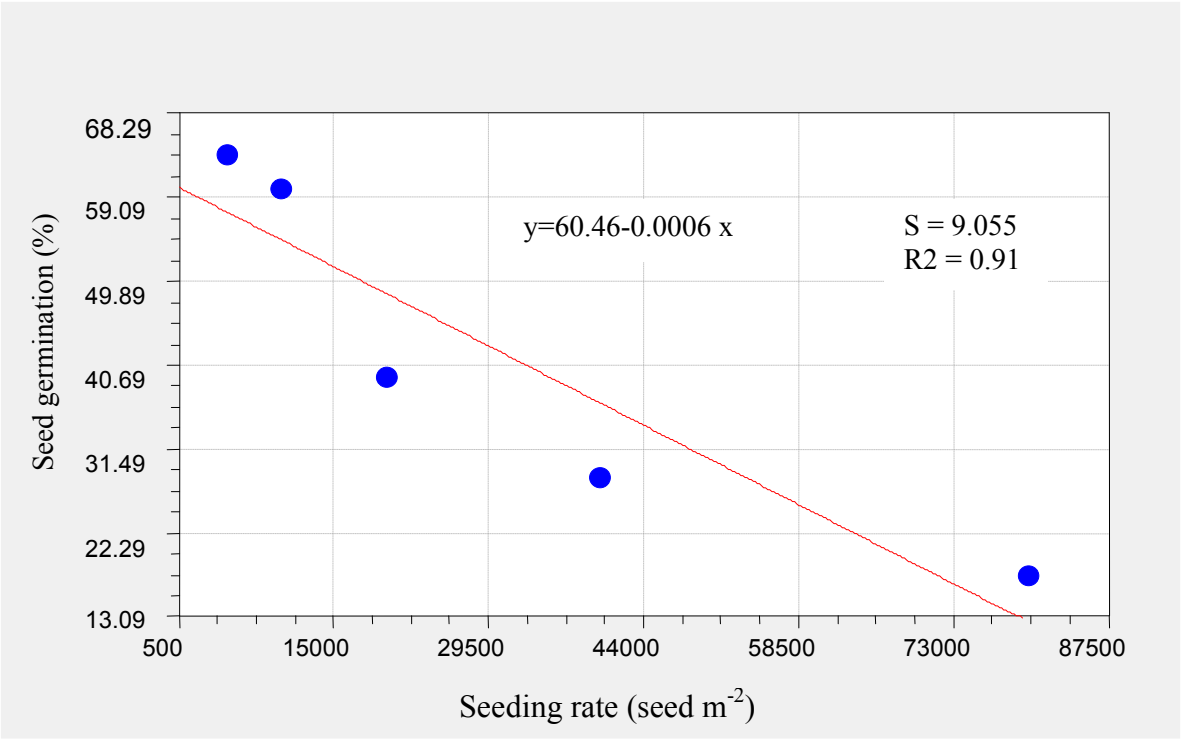

Fig. 4.2. Seed germination as influenced by sowing density of *Brassica juncea* var. Ensabi.

Enquist (2002) reported that the individual plant mortality is a consequence of competition as plants in a stand increase in size. Self-thinning refers that in a competing population of plants, an increase in biomass comes with a reduction in the number of living plants. In other words, when plants grow as individuals, their mean biomass increases and their numbers decrease. Mean individual biomasses at lower density populations were higher than those in higher density. In other words an individual plant's weight increases as the area it occupies increases (**Fig. 4.3**).

Figure 4.3 explains the relationship between total dry matter (g/plant) and plant densities of survivors. According to this result, most increase of plant biomass over time was at the lowest initial sowing density with 5000 seed m^{-2} (**Fig. 4.3**). The plot of the log mean plant biomass (dry weight, calculated by dividing total plant weight per pot by the number of survivors) against the log density of survivors (plant/m^2) showed that total plant weight decreased with time **(Fig. 4.4)**. These reductions commenced in the lower right corner with a large number of plants of small size, and

moving up and to the left as the number of plants decline and the plants grow as the result of self-thinning similar to the finding of Adler (1996).

Based on the results of my study significant differences were observed in the regression models for self-thinning in relationships of the plant biomass and the plant density regimes (**Table 4.2**). This table shows the slope values and 95% confidence limits of slopes. The slope value of -1.5 and it is in line with findings of Xue and Hagihara (1998) based on a much longer observation period on stands of *P. densiflora* and Xue and Hagihara (2002) with results of their investigation on *P. Massoniana.*

The self-thinning slope is more widely accepted, on theoretical grounds, as being -1/5 (or -3/2), as originally suggested by Yoda *et al*. (1963) and subsequent authors, the regression slopes based on the data of this study -3/2 and it is match with finding of Yoda *et al*. (1963) and many of subsequent authors (based on mean biomass as shown in **Fig. 4.4**). The relation of the mean plant biomass and the survivors conforms to the power law with an ideal slope value within the 95% confidence limits of the slopes of the significant models at $p < 0.05$ (**Table 4.2**). The same results showing similar slope structure(s) have been reported by others (Kays and Harper 1974; Westoby and Howell 1986; Zhang *et al.* 2005).

The slope line (**Fig. 4.4**) is -1.5 and the relationship between dependent variable (w) and an independent or explanatory variable (d) is ultimately allometric (Marquet *et al.* 2005). In intra-specific competition, the resultant populations based on differential birth rates and death rates of plants (whether as genet or ramet entities), or number of leaves or branches per plant, (as ramet entities), can regulate individual plant production or population at stable density from a very wide range of initial densities, bringing them to a narrow range of final densities and production per unit area, and it therefore tends to keep density within certain limits (**Fig. 4.4**).

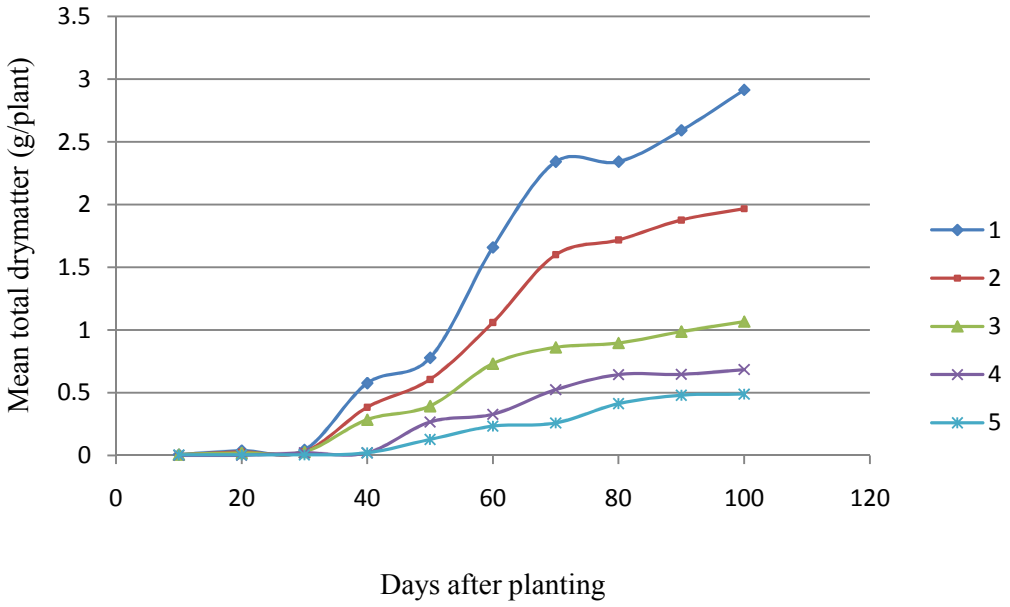

Fig. 4.3. Relationship between dry plant biomass (g/plant) of survivors of *Brassica juncea* var. Ensabi and time (days after planting) at the five initial sowing densities (1-5) of 5000, 10000, 20000, 40000 and 80000 seeds m^{-2}, respectively.

At a population level, slopes of the scaling relationship between average weight of surviving individuals and population density were different. **Fig. 4.4** is a schematic representation of the effects of self-thinning on the number of survival plants and individual plant weights over time in *B. juncea* var. Ensabi populations.

Ogawa (2005) developed a quantitative model to describe the time-trajectory of mean plant biomass and plant density in a self-thinning population when he studied a self-thinning sugi (*Cryptomeria japanica* D. Don) plantation. The model indicted that during the early stages of stand development, when competition has not yet occurred or not severe enough to cause mortality, plant biomass density rises near to vertically, since no high mortality usually occurs and average plant volume increases with no corresponding decrease in stand density.

When interactions among individuals start and the stand grows crowded enough that an increase in the average plant volume cannot occur unless some plants die. At this stage, the time-trajectory in begins to deviates left of the vertical line, referring a reduction in stand density. As competition becomes more severe following its initial bend to the left, the gradient of the trajectory asymptotically approaches and then follows quite closely along straight line, which means that a given increase in average plant volume is matched by a given decrease in stand density. At this final stage of stand development, the gradient of the straight line was assumed to be - 3/2.

Results indicated that each population will start to thin along a line of slope from -2.7 to -1.2 until it reached the maximum standing crop (**Fig. 4.4)**. Mortality during the phase of self-thinning is largely among individuals suppressed by the ensuing growth of neighbours. As the plants grow they begin to interact with each other, and, as a result of this interaction, the smaller, less fit members of the population suffer mortality by increased shading within the canopies of neighbouring plants. This phenomenon may be caused by high shade-intolerant nature of this plant.

It also explained the likely patterns by which populations of *B. juncea* var. Ensabi might increase from an initially very small size of aboveground biomass only to reach the asymptote as time progresses. If a succession of time intervals is taken singly, then each final density can be treated as the initial density for the next time interval.

The analysis of variance and Tukey's Test, indicated significant differences ($p<0.05$) between plants (**Table 4.7**). Results indicated that mean of the measured characteristics were higher at low plant densities. This significant higher mean maybe ascribed to more availability of light, nutrient, water and space at lower densities and accordingly caused more photosynthetic activities among leaves because of in low density treatments the leaf size as mean weight of leaves per plant was greater.

The total dry matter production per unit area was not significantly different at $p<0.05$ (**Table 4.8)**. The total dry matter (m^{-2}) was constant over a wide range of densities (**Table 4.2**) because the reduction in mean plant weight has been compensated following the increase in density (**Fig. 4.5**).

One-way ANOVA and Tukey's Post Hoc Test indicated the significant ($p<0.05$) influence of *B. juncea* Ensabi density on mean shoot weight and leaves per plant. The mean shoot weight and leaves per plant were significantly different at $p<0.05$ (**Table 4.8**).

The relationship between mean shoot weight per plant (calculated by dividing total shoot weight per sample by the number of survivors), and the density of survivors in the populations conforms to the power law. Self-thinning occurred along a line with a slope of -1.62 ($R^2=0.92$, $p<0.05$) with 95% confidence limits of (-2.04, -0.76) an intercept of 10.70, and then deflects from it as dead genets accumulate within the plant populations (**Fig. 4.6, Table 4.3**).

Results also indicated that the leaf survivorship curves of plants at different densities showed different trends. There was significant leaf dry weight in different densities but dry weight leaves of the lowest density was significantly higher than the others. This is maybe because the leaves of the plants in the lowest density able to survive longer and grow old in the canopy than those in the higher densities (**Fig. 4.8**). In lowest density habitat resources, e.g. space, light, nutrients and water, were shared somewhat equitably and this situstion causes their photosynthetic activity falls below that required for balancing respiratory load so that leaves, branches and eventually whole genets begin to die. Consequently, the number of live plants and the proportion of live matter within the population decrease (Donohue and Schmitt 1999). The decline in leaves dry weight and didn't decrease shoot dry weight per unit area during the growing season indicated that the mortality was due to self-thinning (Yoda *et al.* 1963; Asaeda *et al.* 2005).

Normally in practice, few self-thinning populations reach the maximum yields or and self-thinning populations with the slope of exactly -1 are rare (Begon *et al.* 1996; Russell *et al.* 1998).

Some factors in the nature are very important determinant of plant growth. However, lack of some of them might be responsible for inhibitor of growth. Light is one of a few key growth-limiting factors in plants for maximum yield potential, which is highly dependent on growing conditions and on a resource utilization patterns such as canopy light interception and absorption. Plants have evolved specialized mechanisms to grow efficiently in situations of low incident irradiance and increase the interception of light to utilize what little may be available at any given time. These mechanisms and adaptations have been widely studied in the past (Andrews 2008).

Analysis of variance and Tukey's (HSD) Test revealed that the significantly ($p < 0.05$) differences between plant height of *B. juncea* var. Ensabi was observed. Results indicated the tallest plants registered lowest density and the plants in the lowest density was significantly higher than the other densities.

The results also showed that each density regime had a few tall plants while the majority of plants had short height. This tall Ensabi plants dominated the canopy and suppressed the others by exposing them to the relatively inferior light. When plants are competing, larger individuals often obtain a disproportionate share of the contested resources and suppress the growth of their smaller neighbours (Schwinning and Weiner 1998).

Unlike the results of Faravani (2008) in his study on the population biology of *Melastoma malabathricum*, or Baki (1986) in his study on the population biology of *Oxalis corniculata* L. and Nabi (1999) on wrinklegrass, the results of this study differed somewhat.

Further statistical analysis using logarithmic regression on the relationship between plant height (cm) and log density of survivors as a function of time confirmed that the power law prevailed. Self- thinning occurred along a line with a slope of -0.83 ($R^2 = 0.87$, $p < 0.001$), and within the 95% confidence limits to slope -1.09 (or -0.58), with an intercept of 8.12 (**Table 4.5**).

It is generally assumed that self-thinning begins with the smallest individuals in a population and then progressively includes larger ones (White and Harper, 1970, Lonsdale and Watkinson, 1982). It has, however, been suggested that there are exceptions to the - 3/2 power law: high density populations grown in deep shade evidently thin along a slope of -1 rather than - 3/2 (White and Harper 1970; Kays and Harper 1974; Lonsdale and Watkinson 1982).

The regression slopes, however, could change dramatically, and if this happens, it reflects the integrated effects of all external influences such disturbances, grazing, disease, drought, or other stress-inducing factors. Ecosystem processes related to the change in nutrient relations during growth are identified as a prime influence on self-thinning behaviour in natural plant populations (Guo and Rundel 1998; Guiñez and Castilla 2001; Bi 2004).

No significant differences ($p<0.05$) among seed-sowing rates were found in response of intra-specific competition **(Table 4.8).** Ensuing germination and death rates can regulate population to a stable density from a very wide range of prevailing initial densities and can bring them to a narrow range of final densities (**Fig. 4.1**). This therefore tends to keep plant density within certain limits.

With the progress of competition, mean individual of total dry matter of five populations increased, but those in lower density populations increased more than in more crowded populations (**Figs. 4.4** and **4.5**). At the same time, plant densities of all populations decreased, but more plants died in crowded populations than in lower density populations. The results also indicated that the fluctuations within plant biomass of a single plant or unit in populations of *B. juncea* var. Ensabi, whereas plant density decrease leads to parallel decrease in plant biomass.

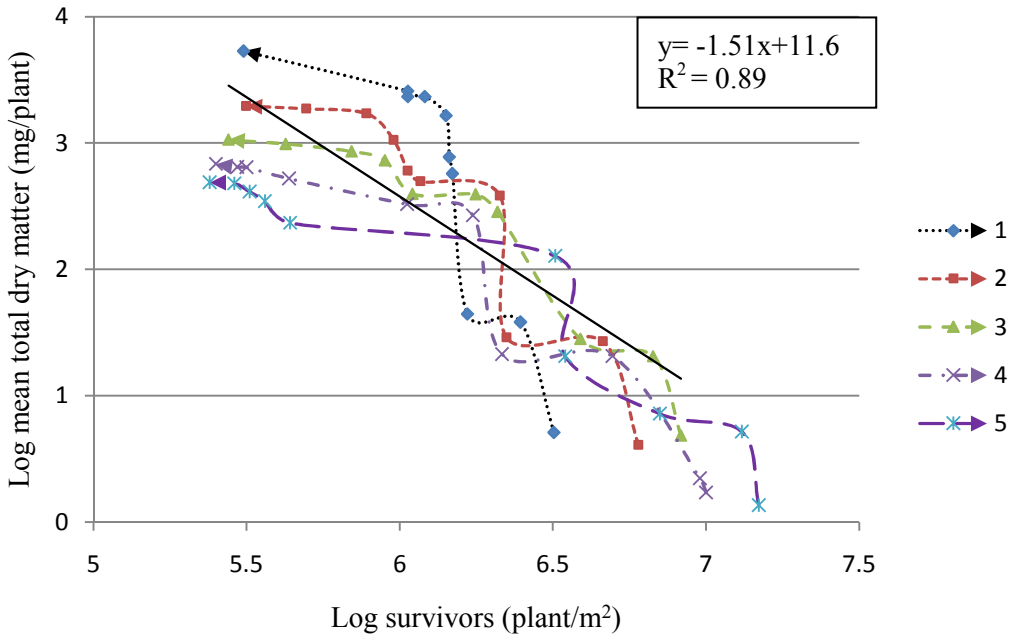

Fig. 4.4. Relationship between log total above-ground dry matter weight per plant and plant density of survivors in populations of *Brassica juncea* var. Ensabi sown at five densities (1-5) with the lines showing populations of the five sowing densities harvested on seven successive occasions indicating the trajectories which, over time (10-100 DAP), these population would have followed. Arrows indicate the directions of the trajectories or the direction of self-thinning. The gradient of the thinning slope was -1.5.

Arguably, the resultant populations from the initial differences in sowing densities can be considered as the stable populations at equilibrium based on no measurable difference in total plant biomass per unit area.

Table 4.2. Gradient and intercept values for thinning lines of populations of *Brassica juncea* var. Ensabi grown under various plant density regimes and plant biomass (mg), calculated by principal components analysis.

Density of survivor plants	Intercept (log k)	Slope (β)	95% Confidence limits to slope Lower – Upper		*Sig F*	Standard error	R^2
1	20.20	-2.86	-4.75	-0.97	0.008**	0.63	0.61
2	13.92	-1.87	-2.91	-0.84	0.003**	0.54	0.69
3	10.94	-1.40	-2.04	-0.76	0.001**	0.41	0.76
4	9.16	-1.21	-1.75	-0.67	0.000**	0.570	0.77
5	6.19	-1.04	-1.29	-0.50	0.000**	0.34	0.85

** and * mean the relationship between two variables in the regression model is significant at $p< 0.01$ or $p <0.05$. The equation of the lines is $\log w= \log k + \beta \log \rho$ where w is plant biomass (mg), ρ is the density of survivors and k and β are constants.

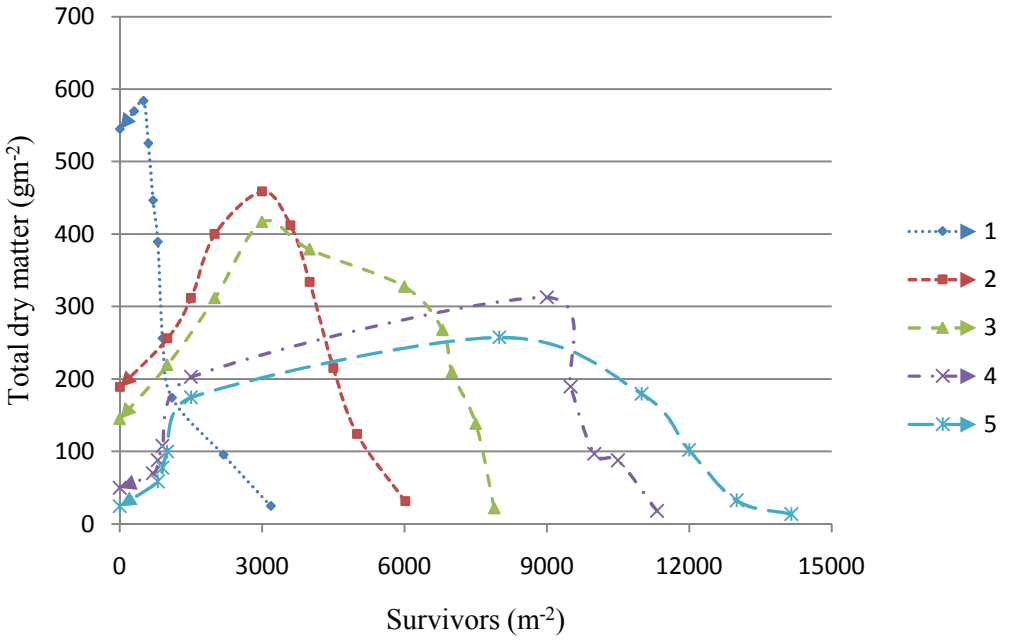

Fig. 4.5. Influence of self-thinning on the total biomass (g/m^2) in *Brassica juncea* var. Ensabi for a period of 100 days after transplanting. Arrows indicate the population dynamics of the growth in survivors over time. Further growth of survivors is balanced by ensuing death of individuals and plant biomass. The five initial sowing densities (1-5) were 3184, 6015, 7891, 11323 and 14154 seed per m^{-2}, respectively.

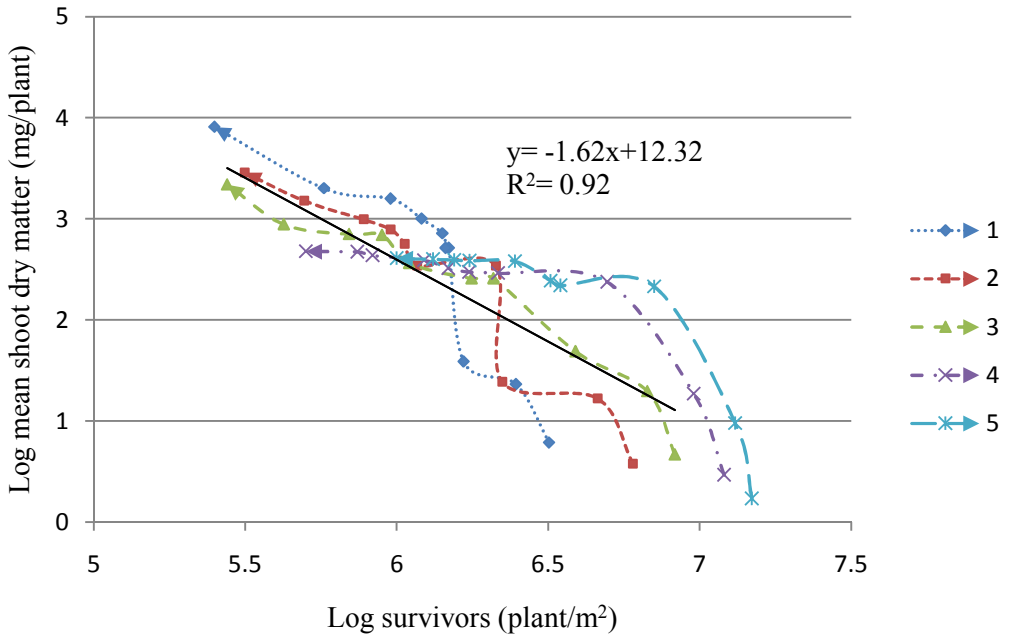

Fig. 4.6. Self-thinning in *Brassica juncea* var. Ensabi populations sown at five densities (1-5) against mean shoot biomass (mg plant^{-1}) with the lines showing joint populations of the five sowing densities harvested on seven successive occasions. They, therefore show the trajectories over time (40-160 DAP) and indicate that these populations would have followed. The arrow indicates the directions of the trajectories, *i.e.,* the direction of self-thinning. The gradient of the thinning slope was -1.10.

Table 4.3. Gradient and intercept values for the thinning lines within populations of *Brassica juncea* var. Ensabi grown under various plant density regimes and mean shoot biomass (mg plant^{-1}), calculated by principal components analysis^{+}.

Density of survivor plants	Intercept (log k)	Slope (β)	95% Confidence limits to slope Lower – Upper		*Sig F*	Standard error	R^2
1	11.42	-1.49	-2.78	-0.02	0.002	0.326	0.77
2	7.36	-1.51	-1.67	-0.35	0.010	0.256	0.86
3	7.65	-0.93	-1.03	-0.65	0.020	0.381	0.88
4	8.10	-0.94	-1.57	-0.29	0.009	0.556	0.68
5	8.83	-1.29	-2.50	-0.09	0.030	0.363	0.63

$^{+}$ Intercept (log k), slope (β), R^2, correlation coefficient, and thinning populations by the reduced major axis using the equation Log w =log k + β log *d*, where w is mean shoot dry weight per plant, *d* is the density of survivors.

During the self-thinning phase, the average nearest neighbour area changes continuously until the entire distribution becomes stable (Li *et al.* 2000). If the succession of time intervals is taken singly, then each final density can be treated as the "initial density" for the next time interval. Thereafter, the biomass increased less and less with each time interval until the population reached its carrying capacity (resources of the environment that can just maintain the population size without a tendency to either increase or decrease (Begon *et al.* 1996). The biomass might follow a "sigmoid" curve due to ensuing competition *vis-à-vis* the onset of competition between survivors in high densities rather than low densities. This is a consequence of the hump in its recruitment rate curve, which is itself a consequence of intra-specific competition. When the log of average shoot weight per plant was plotted against the log of density of survivors for a crowded even-aged plant population, in

such a way that the population's trajectory was held under a line of slope -1.29 ($R^2 = 0.63$, $p<0.05$), and 95% confidence limits to slope -2.5 (or -0.09) with an intercept of 8.83 (**Table 4.3**).

Data showed that there are limited resources available for *B. juncea* var. Ensabi plant growth and that at high densities these are shared among the bigger number of competing individuals. If this is the case, we would expect that provision of extra resources would allow greater growth of individual plants and greater yield per unit area. We have seen that intra-specific competition of *B. juncea* var. Ensabi can, over a period, influence the number of deaths, the number of births, and thus influence the amount of growth and the distribution of biomass within the population. With progressing time, the individuals grow in size, their requirements increase, and therefore compete at an increasingly greater intensity. This, in turn, tends to increase gradually their risk of dying. Thus, the number that survived and the growth rate of the survivors are simultaneously influenced by density.

A slope of -3/2 indicates that in a growing, self-thinning population, mean plant weight increases faster than the decreases in density. A population following a -3/2 thinning line will therefore steadily increase in its total weight (or yield). Eventually, of course, this increase will cease, as yield cannot increase indefinitely. Instead, the thinning line might be expected to change from a slope of -3/2 to a slope of -1 in such a way that the increase in mean plant weight is likely compensated by the decrease in density.

A slope of -1 indicates that further growth of survivors is exactly balanced by the deaths of other individuals. Upon reaching the straightforward asymptote relationship (-1) with the maximum total yield possible, no further increase is possible for the species in question in that environment (Begon *et al.* 1996).

One-way ANOVA analysis indicated that the effect of plant density on the number of leaves per plant was significantly different ($p<0.05$) within 100 days of plant growth (**Tables 4.7** and **4.8**

and **Fig. 4.7**). Plant response to crowding may be mediated by resource availability and/or by a specific environmental cue, with the ratio of red/far red wavelengths (R/FR) perceived by phytochrome (Donohue and Schmitt 1999).

Table 4.4. Allometric relationships between mean leaf mass (mg per plant) for *Brassica juncea* var. Ensabi grown at five plant densities[+].

Density of survivor plants	Intercept (log k)	Slope (β)	95% Confidence limits to slope Lower – Upper		*Sig F*	Standard error	R^2
1	18.14	-2.56	-4.12	-1.00	0.005	0.510	0.64
2	12.70	-1.69	-2.23	-1.16	0.020	0.284	0.87
3	10.70	-1.37	-1.79	-0.96	0.010	0.266	0.87
4	5.90	-0.82	-1.25	-0.19	0.009	0.454	0.68
5	4.67	-0.93	-1.27	-0.19	0.010	0.469	0.63

[+] Intercept (log k), slope (β), R^2, correlation coefficient, and thinning populations by the reduced major axis using the equation: Log w = log k+ β log *d*.

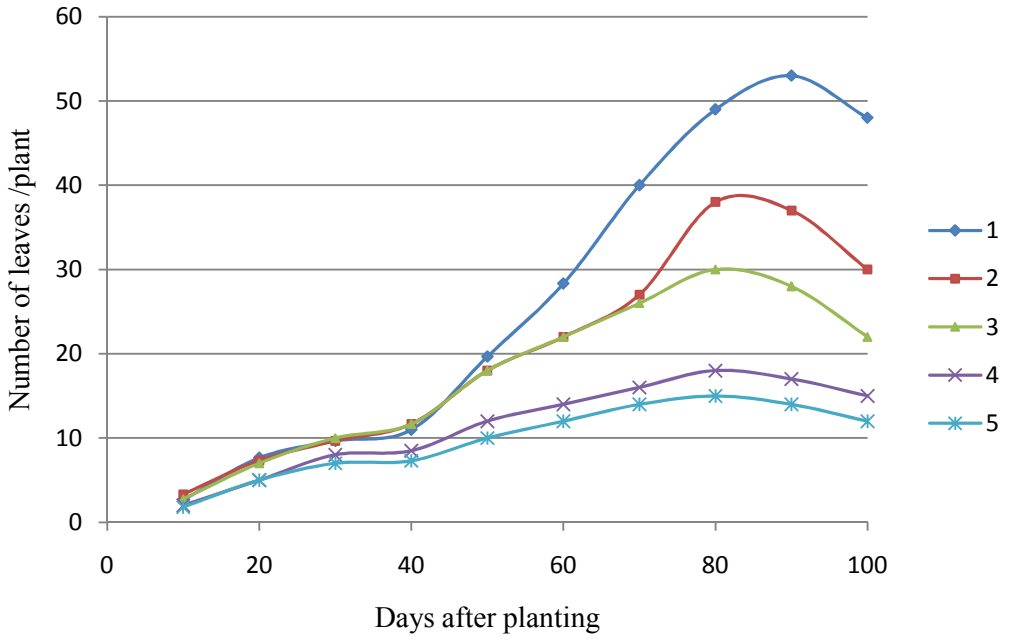

Fig. 4.7. Effect of intra-specific competition on the number of leaves in *Brassica juncea* var. Ensabi against days after planting for five densities of survivor plants. 1, 2, 3 …stand for the five initial sowing densities (19098, 76394, 152788, 229183 and 458365 seed m^{-2}, respectively).

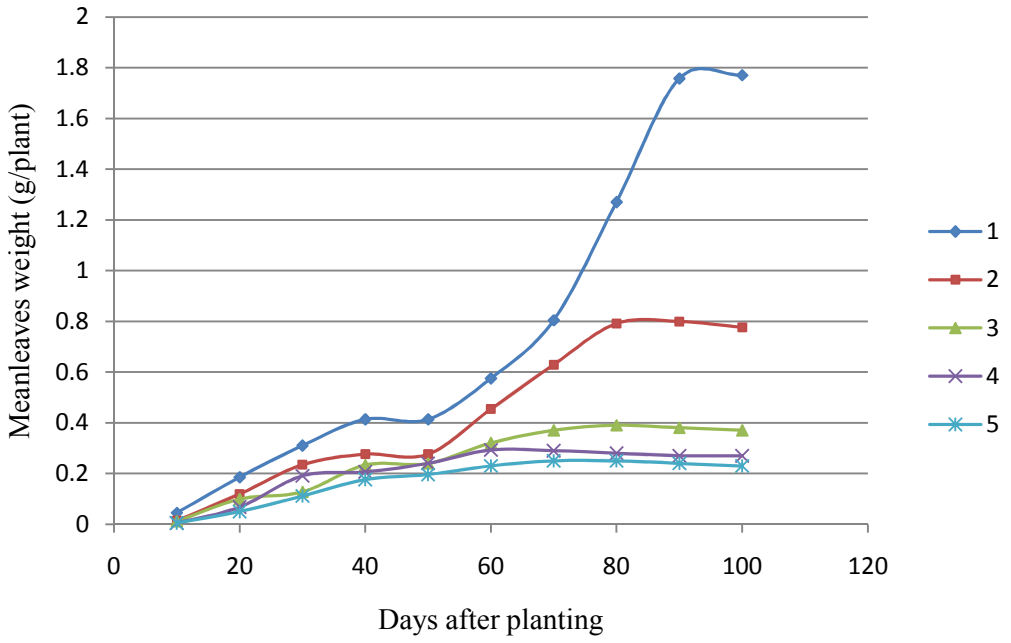

Fig. 4.8. Relationship between leaves weight (g) and plant density of survivors in populations of *Brassica juncea* var. Ensabi at five densities of survivors (1-5).

Based on results of this experiment, density levels strongly affected the slope of the shoot biomass vs. density relationship. Mean plant biomass and shoot biomass were higher at low densities (**Tables 4.2** and **4.3**), which may be caused by more photosynthetic activity in leaves because of leaf size as leaf weight was higher and some recourses, e.g. light, nutrients, space and water are more available at low-density populations (**Fig. 4.8**). In addition, there was significant difference ($p<0.05$) for the number of leaves per plant at different densities within 100 days after planting. As shown in **Fig. 4.7**, there were only slight changes in number of leaves at the lowest sowing densities. This difference also was significant ($p<0.05$) for the weight of leaves at 100 days after planting (**Fig. 4.8**).

The line slope of regression models for leaf weight (**Table 4.4**) indicates changes in plant density, over time, alter the intercept of the thinning line but not its gradient of -3/2, but thinning took place along a line of slope close to - 1. Based on Lonsdale and Watkinson (1982) the thinning slope of plant density population in shade situation shift from -3/2 to -1.

Growth rate is low when there are few leaves, registering either low or intermediately high leaf area index (LAI) values and it is also low with high LAI values, where there is much mutual shading and competition and many leaves may be consuming more in respiration than photosynthesis (Begon *et al.* 1996).

Especially at low densities, growth and hence mean dry weight is roughly independent of density, but with ensuing plant growth over time, density-dependent reduction in growth compensation is denoted by variations in density, leading to achievement of fairly constant dry matter and slope of -1.04 (**Table 4.2**, **Figs. 4.4** and **4.5**).

Table 4.5. Gradient and intercept values for thinning lines of populations of *Brassica juncea* var. Ensabi grown under various plant densities and plant height (cm), calculated by principal components analysis[+].

Density of survivor plants	Intercept (log *k*)	Slope (β)	95% Confidence limits to slope		*Sig F*	Standard error	R^2
			Lower	Upper			
1	12.81	-1.61	-2.64	-0.58	0.006	0.341	0.62
2	9.71	-1.11	-1.47	-0.74	0.000	0.193	0.86
3	8.12	-0.83	-1.09	-0.58	0.001	0.165	0.87
4	5.28	-0.93	-1.58	-0.19	0.001	0.202	0.73
5	4.45	-0.52	-0.93	-0.12	0.002	0.193	0.70

[+] Intercept (log k), slope (β), R^2, correlation coefficient, and thinning populations by the reduced major axis using the equation Log w = log k+β log *d*, where w is plant height and *d* is the density of survivors.

Density levels also significantly ($p < 0.05$) affected the slope of the root biomass vs. density relationship, being greater at low density than at high density (**Tables 4.7** and **4.8**), but in a different way to that seen for shoot biomass, the slope of the root biomass *vs*. density relationship was steeper than that for shoot biomass *vs*. density at the lower density levels, but less steep at high densities (**Tables 4.3** and **4.6**). Root biomass decreased with increasing plant density in all harvests, indicating that root growth was negatively related to plant density (**Fig. 4.9**).

The logarithmic model between different plant survivors at different densities and dry weights of roots conforms to the power law; wherein thinning occurred along a line with a slope of -

0.86 ($R^2 = 0.93$, $p < 0.01$) and within the 95% confidence limits with slope values registering -1.13 (or - 0.46) and an intercept, log k, of 6.83 (**Table 4.6**).

Intra-specific competition can have a profound effect on the number of individuals in a population; but it can also have an equally profound effect on the individuals themselves. This necessarily leads to density-dependent effects on the composition of a population. **Figs. 4.4**, **4.6** and **4.9** show the relationships between survivors and mean biomass (mg/plant), shoot biomass (mg/plant) and root weight (mg/plant) where distribution of sizes within a population has altered because of intra-specific competition. This, in turn, often means that although the numerical size of the population is partially regulated by intra-specific competition, the total biomass is regulated much more precisely. This is illustrated in total dry matter per m^2 as shown in **Fig. 4.5**, in the low-density population where the individuals were relatively large in size (**Figs. 4.4** and **4.6**), while in the high-density population they were relatively small in size. Overall, however, the total biomass (m^2) was roughly the same at all densities. In fact, this is a pattern that has been called "the Law of Constant Final Yields" (Kira *et al.* 1953).

No differences were detected in the ratio of shoot to taproot growth at $p<0.05$ between plant densities over 100 days after planting (**Table 4.8**). Our data indicative that in *B. juncea* var. Ensabi, re-allocate biomass from belowground to above-ground plant parts when light availability was reduced (with increasing plant density) through above-ground competition was not occurred and it was showed the optimal partitioning theory did not prevail in our results. However, it is more difficult to argue about it. Morris (1999) reported plants at the higher density population and at the lower fertility levels allocated more biomass to root growth.

Infer the intensity of above-ground and below-ground competition from evidence such as changes in the allocation to and the dimensions in space of the resource-acquiring organs of individual plants (Lonsdale and Watkinson 1983; Morris 1999), and changes to population-level

measures such as leaf area index, or size inequality can help to arguments about the relative strength of shoot and root competition in self-thinning populations.

These results are in line with the results of Faravani (2008) who studied the population biology of *Melastoma malabathricum*, but are in contrast with the lower root: shoot ratios obtained under reduced light intensity in a number of other studies (Wilson 1988). However, they were in agreement with Casper *et al.*'s report (1998) on *Abutilon theophrast*, as density did not affect root: shoot ratio, the partitioning of biomass between fine roots and main roots, fine root length, or root specific length; in other words, above ground competition does not alter biomass allocated to roots.

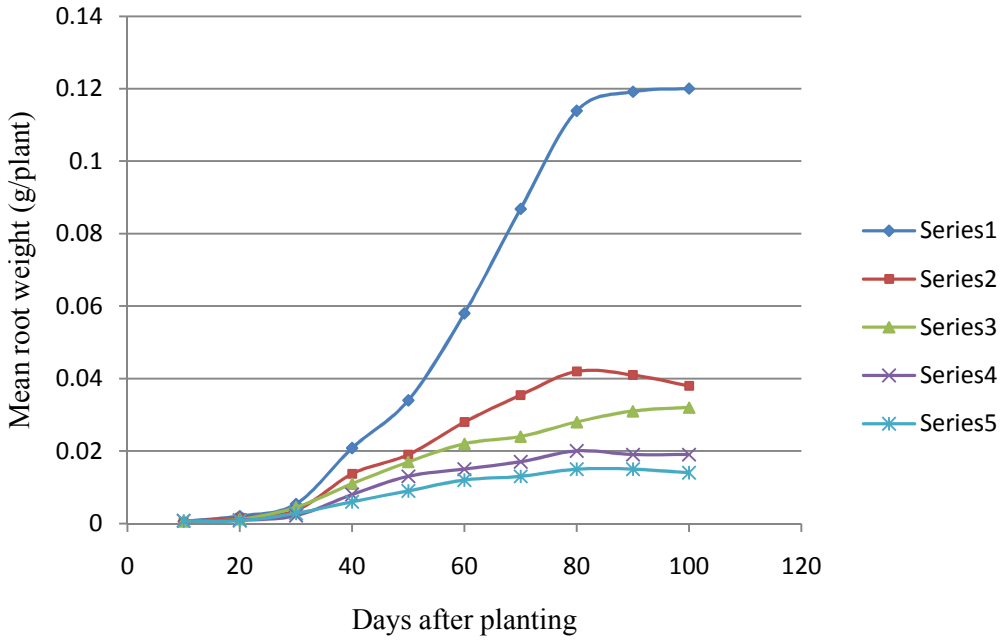

Fig. 4.9. Relationship between root weight (g) and plant density of survivors in populations of *Brassica juncea* var. Ensabi at five densities of survivors (1-5).

Table 4.6. Gradient and intercept for the thinning lines of populations of *Brassica juncea* var. Ensabi grown under various plant density regimes and mean root biomass (mg/plant), calculated by principal components analysis[+].

Density of survivor plants	Intercept (log *k*)	Slope (β)	95% Confidence limits to slope Lower	Upper	*Sig F*	Standard error	R^2
1	18.05	-2.65	-4.12	-1.20	0.003	0.483	0.68
2	12.65	-1.80	-2.26	-1.35	0.020	0.240	0.91
3	10.33	-1.43	-1.73	-1.12	0.001	0.196	0.93
4	7.20	-0.99	-1.23	-0.55	0.001	0.256	0.88
5	6.83	-0.86	-1.13	-0.46	0.010	0.191	0.93

[+] Intercept (log k), slope (β), R^2, correlation coefficient and thinning populations by the reduced major axis using the equation Log w=log k+ β log *d*.

Plasticity in morphology and physiology acts to reduce the degree of size asymmetry in competition (Schwinning and Weiner 1998). This property in biomass allocation, root morphology and root distribution pattern was found to be an important adaptive mechanism to acquire nutrient resources (Xie *et al.* 2006; Xie *et al.* 2007). We expected root: shoot ratios to be higher at the low-density population and decrease under high density for *B. juncea* var. Ensabi because of the disproportionate advantage of aboveground size in competition for light (Weiner *et al.* 1990). One possible explanation of the lack of root: shoot response in *B. juncea* var. Ensabi is that taproots provide structural support and strong root anchoring. They could be increasingly important as plants stem-to-leaf ratio increase in response to aboveground competition (Casper *et al.* 1998).

The results of crowded population on root weight (**Table 4.6**) showed that increased root competition could lower the slope and/or intercept of the self-thinning line transversed by plant populations. Root: shoot ratios were remarkably constant over the broad range of plant size achieved in the five densities. Thus, these plants do not exhibit above- versus belowground biomass trade-offs in their ability to compete for light versus belowground resources.

In summary, results support the concept of the competition-mediated self-thinning rule among competing plants. The different self-thinning power value is a density-mediated plant response to the resource utilization and sensitivity to stress. Because regression slopes are affected by multiple factors, they can vary greatly among species and habitats. Regression analysis could be very helpful in identifying the presence of other factors that affect the development of ecological communities. More well controlled experiments should be carried out in order to identify the more accurate values between −3/2 and −4/3 or -1.

Considering all morphological attributes of the different growth forms suggests that growth form differentiation may be a plastic response to increasing levels of density stress. For example, increased density in plant populations commonly resulted in decrease in plant size as a result of increasing competition for limited resources (Harper 1977). Likewise, the decreased allocation of resources to sexual reproduction is a common response to high levels of intra-specific competition (Harper 1977).

Table 4.7. Multiple comparisons with Tukey's (HSD) test at $p<0.05$ on selected plant growth parameters of *Brassica juncea* var. Ensabi[+].

Plant density (m^2)	Density of survivor plants (m^2)	Mean biomass (g. $plant^{-1}$)	Root weight (g. $plant^{-1}$)	Leaves weight (g. $plant^{-1}$)
1	1397 b	0.37 a	0.15 a	0.19 a
2	1795 b	0.16 b	0.09 b	0.08 b
3	2377 b	0.09 b	0.07 b	0.03 b
4	2940 b	0.07 b	0.05 b	0.02 b
5	3855 a	0.05 b	0.04 b	0.01 b

[+]Values followed by similar letters within the same column are not significantly different at $p<0.05$.

CHAPTER 5

THE INFLUENCE OF SPACING GRADIENTS ON GROWTH AND SELECTED MORPHOLOGICAL CHARACTERS OF BRASSICA JUNCEA VAR. ENSABI

5.1 INTRODUCTION

Ecologists often write of competition for physical space. This should mean that space is in limited supply and that this reduces plant growth (Wilson and Harder 2003). The discussion of competition for space may be justified, though it is misleading and obscures the differences between resources in their mechanisms of competition (Wilson 1988). If the available space is limited for a plant, then plant competition occurs for light, water and nutrients. Each individual plant should grow until its weight is proportional to the size of its immediate, available space. This has led to the development of neighbourhood models of plant performance (Franco 1985; Ramstad and Hestmark 2000).

The productivity of short rotation biomass plantations will vary with many factors including the spacing utilized in the planting. The nature of energy plantations, where productivity is to be maximized in an extremely short rotation, necessitates determination of optimum planting density.

In recent years, there has been growing interest in developing methods for the study of plant interference. Such growing interest in developing methods for the study of plant interference is important that plants are arborescence. Studies on the effects of inter- or intra-plant spacing on the growth of plants have been conducted in both annual crops and trees to achieve the maximum possible yields. That being the case, plants cannot escape the effects of interference, which considered important for a plant of its fixed position. Because plants cannot escape the effects neighbours 'movement' is restricted to the plasticity of growth of the population of meristems present in different parts of the individual plant (Franco 1985; Franco and Harper 1988).

Nelder (1962) published a description of a series of systematic experimental designs specifically for studying plant-growing space and planting alignment in vegetables at Rothamstead Research Station in the UK. The designs consist of a grid of points, each representing the position of a plant and having the property that the growing space per plant

and/or the rectangularity of the space available to each plant changes systematically over the grid. Fan design, is also known as the Nelder's Wheel, is a systematic planting design in which plants or trees are planted at the intersections of circular arcs and linear spokes. It is an alternative to arbitrary assignment or model estimation of a critical radius to empirical test a range of competition-free neighbourhood sizes on the performance of trees in benchmark ecosystems.

Nelder experiments have been restricted predominantly to the study of competition among individuals of single spices (Nelder 1962). This design usually consists of a grid of plants often planted as an arc or circle. The area per plant or the amount of space available to each plant changes in a consistent manner over the different parts of the grid. The circular plots developed, by Nelder make it possible to avoid the difficulties of the rectangular plots and still study density response over a wide range.

In general, Nelder's plots allow many different planting densities to be examined in a single plot. This is frequently more efficient than planting a different plot for each planting density and also for studying the effect of inter-plant spacing on the growth of individual plants in a reduced space and with equal or almost equal sample size (number of plants) for each spacing (Simard and Zimonick 2005). It is possible to examine the effect of different planting geometries in a single Nelder's plot (**Fig. 5.1**).

With Nelder's experiments, studies on a wide range of growing space and alignment treatments, whereas randomised block spacing experiments are concerned solely with growing spaces, can be conducted. The range and number of treatments were likewise reduced in the randomised block design experiment (Vanclay 2006). Nelder (1962) pointed out that this assumption might be valid in gradients whose steepness (the difference in spacing between successive arcs defined by the parameter *"a"*) is not too great. Fan design may be a useful design for studying the way in which neighbour effects is transmitted in populations of organisms that cannot move away and escape from each other.

The competition threshold concept defines the density of neighbors at which competition occurs and target plant growth is limited (Simard and Zimonic 2005). In theory, plant performance in neighbourhood models should enable prediction on the results obtained from such gradients. In practice, neighbourhood models of plant performance assume a monotonic relationship between plant yield and inter-plant spacing, and such a relationship would fail to account for the regularity in the pattern of dominance and suppression of prevailing plants generated in such designs. It is known that when plants compete, they develop a hierarchy of dominance and suppression. The representation of these processes in a model that is mathematically tractable and ecologically meaningful is a big challenge (Franco and Harper 1988; van Wijk 2007).

The emergence of spatial pattern and the theory of survival analysis when individual plants are equally spaced have been studied by means of the statistical technique known as spectral analysis (Franco and Harper 1988; Hong *et al.* 2004; Arrieta and Suárez 2005). The spatial pattern develops when they are equally distributed or when plant competition occurs irrespective of the planting regime (Franco and Harper 1988; Gallardo and Parama 2007). Harper (1977) suggested that 'edge effects' and resource pre-emption are in essence the same phenomenon.

Plant–plant interactions are an important part of the mechanisms governing the response of plant species and communities in natural systems (Brooker 2006). The importance of competitive and facilitative inter-plant interactions in plant community structure, diversity, development and the formation of pattern in spacing gradients were well recognized. A number of studies have been done on the transmission of competition effects as plants along the gradient start interfering with their neighbours at different times (Harper 1977; Simard and Hannam 2000; Simard and Zimonick 2005). Increasing competition through increasing neighbour density and proximity has been shown to reduce target tree growth (Harper 1977), but it may also affect carbon allocation patterns (Davidson *et al.* 2002) and tree morphology (Simard and Zimonick

2005). Increasing neighbour density resulted in reduced crown length, branching, and plant height (Simard and Zimonick 2005; Lieberman and Lieberman 2007).

Due to inter-plant competition, there is a negative correlation in the growth of neighbouring plants or plant size with increasing available area. Canopy sizes of plants increased with distance to the nearest con-specific neighbour, which suggests that neighbour interactions negatively affected plant sizes. Intraspecific and interspecific has been inferred from correlation between nearest neighbour distance and plant size. On the edge of the design, plants have more space to develop and behave as dominants over their immediate neighbours; this dominance releases their neighbours' neighbours from competition, thus producing a periodic distribution of dominant and suppressed plants (Franco and Harper 1988; Fordyce *et al.* 2000; Maron and Vila 2001; Schenk *et al.* 2003). The threshold neighbourhood size between neighbouring plants is influenced by the critical distance from the target plant at which neighbours start to limit the targeted neighbour plant performance. Defining the competition arena for free-growing trees is an important component in forest vegetation management. This is determined by either successively removing or adding neighbours at increasing distances from the target plant, and then measuring target responses (Lieffers *et al*. 2002; Simard and Zimonick 2005).

To develop strategies for manipulating vegetation, accurate estimates of the spatial gradient of neighbour competition, as well as the zone of maximum release, are required. The ensuing experiment in which *B. juncea* var. Ensabi was grown in a spacing gradient, was designed with the following objectives:

1. To model the effect of different initial spacing of plants on growth, branching and other characteristics in a single Nelder's experiment;
2. To model the confidence bands for the predicted mean values for all plant characteristics at the 95% level of confidence;

3. To assess the effect, if any, of different initial plant alignment on plant, determine neighborhood size effect on mortality, growth, and morphology of *B. juncea* var. Ensabi*;*
4. To note the emergence of spatial pattern in plant populations;
5. To study the interference spacing gradients effect on modular organisms; and identify the critical radius for each response variable.

5.2 MATERIALS AND METHODS

We evaluated intra-specific competitive interactions in *B. juncea* var. Ensabi plants during the planting to harvesting in Rimba Ilmu, University of Malaya to access the response variables in relation to the critical radius of competing plants. Seeds of *B. juncea* var. Ensabi*,* were sown in wooden boxes previously filled with garden soil of Malacca series in an insect-proof house. The plants were subjected to 12 hours of natural sunlight outdoor (mean midday radiation of 1834 μmole photon m^{-2} s^{-1}), and 367 μmole photon m^{-2} s^{-1} inside in insect-proof house, and mean ambient temperatures of 33±2^{o}C (day) and 25±2^{o}C (night) at Rimba Ilmu, University of Malaya on October 2, 2007. One hundred twenty six young uniform seedlings of *B. juncea* var. Ensabi of approximately 2.5 cm height and 4 leaves including the cotyledonary leaves were selected randomly and transferred to a 25 cm deep soil bed (1.4 m × 1.4 m) containing the same soil compost enclosed in a wooden frame 30-cm high on December 28, 2007. Care was taken to ensure that the scar left by the cotyledon leaves was positioned at surface ground level. This gave a standard point from which to measure plant height, stem diameter 1 cm above it, and separation of aerial and root parts at harvest.

5.2.1 The experimental design

In design type Ia, the area per planting point location increases outwards from the centre. The rectangularity of the growing space is constant over the whole grid. Nelder (1962) specified that the shape of the growing space available for each plant is the same throughout the plot and

that plants on different spokes but at the same radius shall have equal growing space, so the following geometric progression could be derived (Redmond 2003).

The position of each plant was located in a polar co-ordinate grid with arcs and spokes (fan design type I; Nelder 1962) with the following parameters:

$$r_n = r_0 \alpha^n$$

where: r_n = the radial distance of the n^{th} plant in the spoke (n = 1, 2, .. N);

r_0 = the radial distance of the starting plant in each spoke;

α = the constant determining rate of change in growing space.

Angular distance between plants: $\Theta = 20^o$ or 0.349 radian.

One more parameter of this fan design, called eccentricity (R), is defined as the symmetry,(or departure from it) of the space available to each plant, and is characterized by the ratio between inter-arc distance and inter-ray distance. Clearly, when this quotient is equal to one the space available to each plant is symmetrical.

The position of each arc is defined by an exponential function ($r_n = r_0 \alpha^n$) that describes its distance from the origin of the grid (r_n) to the n^{th} arc. The function has two parameters: the distance, somewhat arbitrarily defined, of the first arc (r_0) and a coefficient (α) that defines the rate with which the distance between arcs increases (**Fig. 5.1**). This parameter is necessarily greater than one.

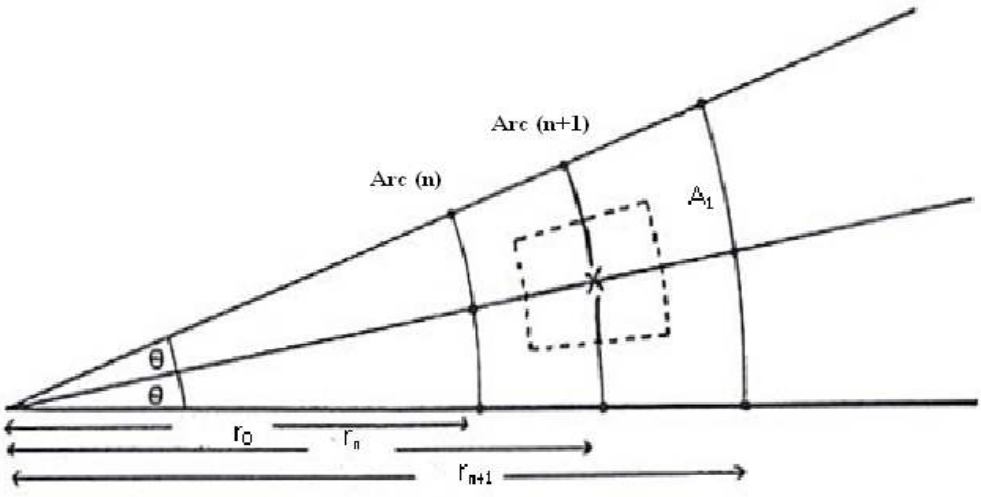

Fig. 5.1. Details of the Nelder's design type Ia (Nelder, 1962).

While n=0, N+1 defines a single guard row/arc at each end. Similarly, the experimental spokes are identified by m = 1, 2...M, while m = 0 and M+1 represent guard rows at the sides where the design does not run through 360^0. The number of treatment radii or growing spaces examined, r_n and α, the design is also defined by the angle between the spokes (θ), measured in radians (**Fig. 5.2**). Growing spaces is also defined by the greatest planting density (A_N) and the smallest planting density (A_1) growing space, and the rectangularity or eccentricity (R) of the planting arrangement (symmetry) of the space available to each plant, and is characterized by the ratio between inter-arc distance and inter-ray distance. Clearly, when this quotient is equal to one, the space available to each plant is symmetrical.

Specifying parameters: A_1, A_N, R and N in the design type

- A_1 = the smallest planting density;
- A_N = the greatest planting density;
- R = the rectangularity of the planting arrangement;
- N = the number of growing spaces examined.

Having chosen 0 to be 20° or 0.349, and in order for the value of eccentricity to be equal to one, the the parameters were calculated from the formulae presented (Nelder 1962; Franco 1985; Redmond 2003) as:

I. The constant α that determining the rate of change in growing space is calculated using:

$$Log_{10}\alpha = \frac{\left[Log_{10}A_N - Log_{10}A_1\right]}{2N-2}$$

II. The angle (θ) between the spokes is calculated by:

$$\theta = R(\alpha^{0.5} - \alpha^{-0.5})$$

III. The radial distance (r_0) of the starting planting position in each spoke is calculated (**Fig. 5.2**):

$$r_0 = \sqrt{\frac{2A_1}{\theta\left(\alpha^3 - \alpha\right)}}$$

IV. The radii of the remaining arcs (r_n) are calculated using:

$$r_n = r_0\alpha^n$$

V. Finally, the area (m^2) available to each tree (A_n) is calculated by:

$$A_n = \frac{{r_n}^2\theta\left[\alpha - \alpha^{-1}\right]}{2}$$

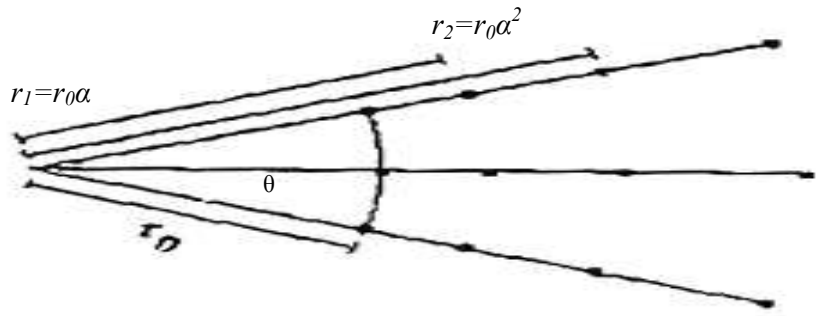

Fig. 5.2. A schematic representation of Nelder's design (drawn from Redmond 2003).

The minimum and maximum areas per plant were decided to be 0.01 m^2 and 0.581 m^2 respectively, and the distance to the first arc as $\mathbf{r_0}$ =5.71 cm with α =1.4. The allocated space per plant and calculated corresponding density, the number of plants per meter square, are shown in **Table 5.1**.

Arcs were numbered from 1 to 7. Plants in each arc were numbered clockwise. Plants were arranged in a triangular array superimposed on the 'rectangular' fan design, giving nineteen plants per arc (**Fig. 5.3**).

The available space necessarily would be greater for plants at the edge of design. Since this arrangement of plants produced a strong "edge effect", statistical analyses were usually done by excluding plants that have died previously in each recording date and plants in edge arcs 1 and 7. Additional statistical analysis was be made on the whole data set considering only those plants that remained alive during the whole duration of the experiment to see whether the edge plants are responsible for the formation of spatial pattern (Franco 1985).

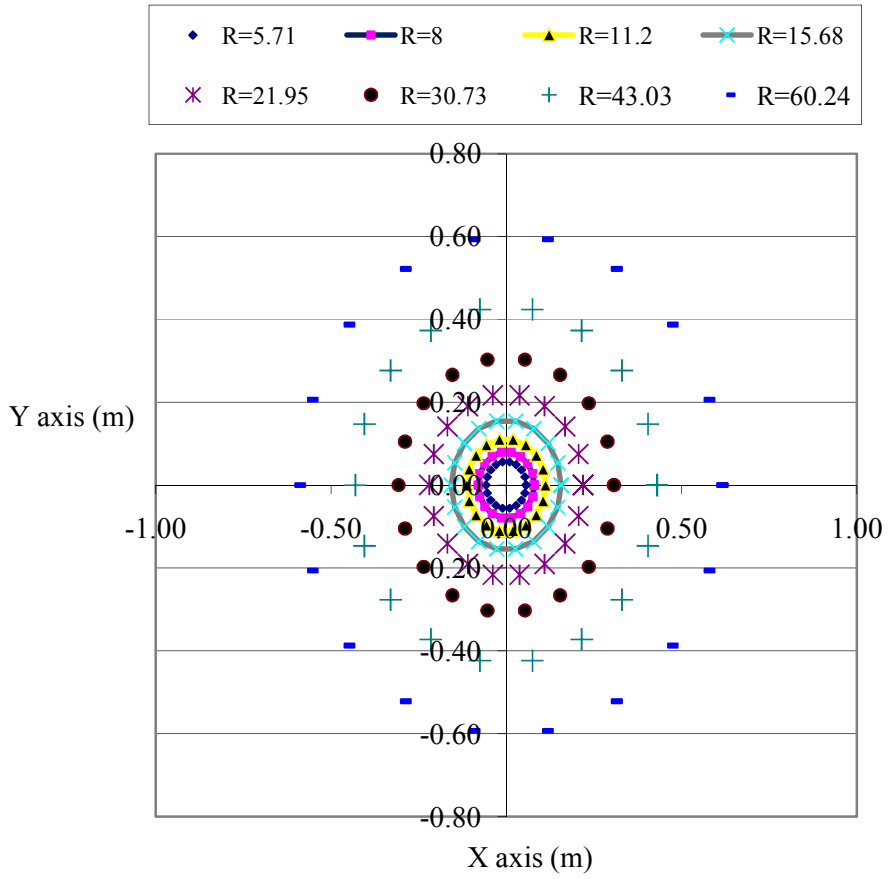

Fig. 5.3. Schematic diagram of the Nelder's design type Ia denoting the gradients and bird's eye view of the arrangement of fans on the soil bed used to investigate the effect of inter-plant spacing on the growth of individual plants *Brassica juncea* Var. Ensabi The location of each plant illustrates the ordering of arcs (1-7) and spokes (1-18) for 126 plants.

Table 5.1. Initial available space per plant and calculated density in experimental layout.

Arc number	Radius from origin (cm)	Area per plan (m^2)	Density (plants/m^2)
1	5.71	0.010243	98
2	8.00	0.020106	50
3	11.20	0.039408	25
4	15.68	0.07724	13
5	21.95	0.151363	7
6	30.73	0.296671	3
7	43.03	0.581691	2

5.2.2 Measurement techniques

Measurements of plant growth parameters were done on four randomly selected spokes. Growth parameters, namely plant height, number of pods per plant, number of seed per pod, and pod lenght, leaf numbers per plant; were recorded in 10, 20, 30, 40, 60, 70, 80, 90, 100 and 110 days after planting. Plant height and stem diameter were recorded using a metallic measuring tape and a Vernier calliper, respectively.

Plant were harvested 3 months after planting and they were dismembered at ground level and separated into stem, leaf, and root components and dried in an oven at 50°C for one week. Finally, these plant components were weighed.

5.2.3 Data analysis

The respective growth data were analyzed with ANOVA and regression analyses were performed where appropriate. The process of finding the best fit was done by Curve Expert 1.3 by comparing the data with each model to choose the best curve. The appropriate model to use

depends on the nature of the data and the underlying hypothesis associated with the cause or function of the phenomenon described by the response variable. The independent variable (x) was growing space value and is made up from two distances: the distance between plants on the same arc and distance between plants on the same spoke. These three values are closely correlated; therefore, only the growing space (cm^2) was used in the analysis as an independent variable **x** with plant height, biomass,…as dependent variables y (Redmond 2003). The (x, y) data can be modeled using a toolbox of linear regression models, nonlinear regression models, interpolation, or splines; linear and nonlinear regression is the most common technique applied in growth model parameter estimation (Garcia 1988).

One of the widely used relationship between **x** and **y** take the form $y = ax^b$ where ***y*** is some response or dependent variable, **x** represents an independent or explanatory variable, **a** is normalization constant and b is the scaling exponent, and where 'b' has been shown to be -3/4 both on empirical and theoretical ground systems. This power-law regression models are commonly used to analyze ecological systems (Marquet *et al.* 2005). This model can be linearized by taking logarithns to the base 10, i.e.

$$\text{Log } y = \log a + b \log x.$$

If Y=log y, α = log a and X= log x then the standard linear equation is:.

$$Y = \alpha + bX$$

Non-linear regression analysis is also suitable yield-density functions by Nelder (1962). The ANOVA and spatial auto-correlation method were evaluated for Nelder's wheel results (Franco and Harper 1988; Nabi 1999; Redmond 2003; Faravani 2009). Application of spatial methods demonstrated the complexities of modelling with large numbers of irregularly distributed observations, and these are due to the wide range of treatments in Nelder's designs, regression models may be developed, which can be used confidently.

The measure ρ of linear relationship between two variables X and Y is estimated from $(x_1, y_1),.....,(x_n, y_n)$ by the sample correlation coefficient **r**, where

$$r = \frac{\sum x_i y_i - \frac{\sum X_i \sum y_i}{n}}{\sqrt{(\sum x_i^2 - \frac{(\sum X_i)^2}{n})(\sum y_i^2 - \frac{(\sum y_i)^2}{n})}}.$$

To test the null hypothesis $H_0 : \rho = 0$ against the alternative hypothesis $H_a : \rho \neq 0$, we compute the test statistics:

$$T = \frac{r\sqrt{n-2}}{\sqrt{1-r^2}}$$

that follows a *t*-distribution with n-2 degrees of freedom.

5.3 RESULTS AND DISCUSSION

To determine whether the complete data set with the edge data (arc 1 and 7) included could be used in our statistical analysis we used the one way ANOVA.

Based on one way ANOVA, no significant differences between the two data sets at the significant level $p<0.05$. The pattern of arc dominance and suppression appears more clearly, when one considers the whole data set. Therefore, to get a better view of the trend of the variables, our data analysis were conducted with the "edge" arcs 1 and 7 included. They were directly responsible for the formation of spatial pattern and their inclusion is necessary in the assessment (**Figs. 5.4** and **5.5**).

5.3.1 Effect of growing space on plant height

Results indicated a highly significant differences (p<0.01) was observed between different spacing and plant height (**Fig. 5.4**). The range of mean height of all treatments in this experiment that remained alive until maturity increased with both time and spacing.

The ANOVA estimation of variance components were carried out for individual experimental data from four spokes in 7 arcs in order to assess variations of the plant height among individuals along space gradients. Results documented plants at the closer spacings, were taller than those at wider spacings from 10 DAP until 40 DAP; instead at maturity, plants in wider spacing recorded significantly taller than those. The multiple comparisons with Tukey's Honestly Significant Difference Test (HSD) shows the shortest plant height was found in Arc 1 and it was not any significant differences recorded between Arc 1 and 2 and also noted the tallest plans were recorded in Arc 7 and plants in this Arc significantly taller than those (**Table 5.4**). The increase of plant height with spacing did not occur monotonically (space was pre-designed & fixed, there was no time-mediated increase/decrease as such); instead, the fluxes in plant height figures showed a series of ridges and furrows which suggest that some arcs dominated over neighbouring arcs.

Plant height showed a gradual increase at 30 days after planting (DAP) and high increase at maturity. Therefore, the degree of interference is not a monotonic function with respect to inter-plant spacing. The greatest neighbour effect in the fan design occurred in arc 1 and the smallest neighbour effect were found in arc 7. The linear fit model of logarithm plant height (y) against logarithm plant density (x) determined a negative relationship of the neighbour effect in the development of the stand and, given its regularity when one considers the height of the plants, suggests that any differences in total height determine the differential success of neighbouring individuals (**Fig. 5.5**).

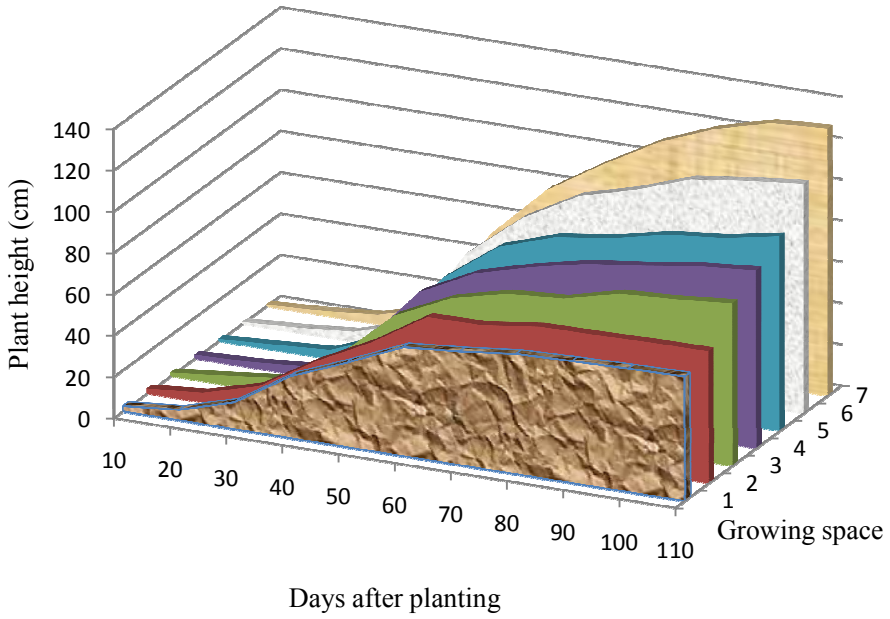

Fig. 5.4. Response of *Brassica juncea* var. Ensabi to a range of inter-plant spacing through time as measured by the mean total height of plants that remained alive until the end of experiments in four selected spokes and arcs 1 to 7 at 11 measured times for 110 days after planting date (DAP).1,2,3…7-arcs with different spatial arrangements.

Fig. 5.5. Linear logarithmic model for plant height value (cm) of *Brassica juncea* var. Ensabi as a function of plant density (m^2).

The regression model is strongly significant at p <0.01 with a good coefficient of determination (R^2 = 0.82) and the t-test for the coefficient of determination showed a significant difference at p<0.01 (**Table 5.2**). It showed that presence/absence of plant densities studied was significantly affected on plant height. Plant densities and relative distance/spacing between neighbouring individuals affect plant height and these effects were more pronounce with ensuing competition through time. In effect, these were translated into individuals with hierarchical differences in plant height with those in the arc 1 in the centre displaying significantly shorter stands than those in arcs 2, 3, 4, 5, 6 and 7 and in that ascending order of magnitude.

Based on **Fig. 5.5**, the relationship between growing space and plant height was highly significant. The regression model notes that closer growing spaces are associated with reduced growth of mean plant height in *B. juncea* var. Ensabi**.** The sharp decline in mean height growth at the closer growing spaces is not widely found in the literature, except where extremes of spacing are studied (Gilliland 1981; quoted by Redmond 2003). However, the small growing spaces employed in this study could not be considered as 'extreme'. According to Joyce *et al.* (2002), height growth is mainly a function of site, species and genetics.

There is a plethora of data and information or documented evidences on the effect of growing space on plant height growth. They indicated an increase in mean plant height of the total population with increased growing space per plant (Albaugh *et al.* 2006). On the contrary, some researchers reported a decrease or no significant change with increased growing space per plant (Srivastava *et al.* 1999; Mehari and Habte 2006).

Table 5.2. Regression analysis on plant height and number of leaves per plant (y) of *Brassica juncea* Var. Ensabi in growing space (x) for linear logarithmic model (L): Log y= log a+ b log x.

Mean squares			
Source	df	Plant height (cm)	No. leaves/plant
Regression	1	0.345**	0.567**
Model		L	L
Residual	26	0.003	0.007
A (constant amount)		2.150	2.228
B (coefficient of the variable)		-0.192	-0.247
t- value for r^2		-33.801	-28.294

** significant F-test at $P < 0.01$

5.3.2. Effect of growing space on the growth of leaves and pods

Based on **Table 5.3**, the growing space strongly affected the number of leaves and pods/plant, seed/pod, pod length and stem diameter. It is clear from the results of this experiment; increasing plant density influenced the vegetative growth characters of *B. juncea* var. Ensabi. This was seen with differences in mean number of leaves /plant, pod/plant, pod length, seed/pod and stem diameter as the consequence of spatial stress experienced by these vegetative organs (**Tables 5.3** and **5.4**).

Regression analyses showed a strong linear logarithmic model relationship between the number of total leaves in plants and plant densities (R^2=0.88 and p <0.01). It was observed that stands growth exhibited ensuing increase at higher spaces (**Table 5.2** and **Figs. 5.6** and **5.8**).

According to results presented in **Table 5.2,** there was a strong relationship between the number of total leaves in plant, age (days after planting) and growing spaces or plant density (**Figs. 5.6** and **5.7)**. This was attributed to the fact that the number of leaves were inhibited by limited resources as the result of ensuing competition by neighbours, thereby reducing stand growth of plants. The HSD tests revealed that the arc 1(lowest density in the experiment) produced the highest number of leaves to compare with all spacing growth. Less competition for nutrients, water, and light and increased than airflow and light could be possible explanations for the greater leaf number at lower population density treatment. Results also indicated there were no significant (p<0.05) difference in the number of leaves among plants or along arcs 6-7 (**Table 5.4**). It was documented the increased rate of interference zones of competing plants leading to a relatively inferior light environment and ensuing competition, hence the resultant plant spacing greatly affects leaf area, light interception, and canopy apparent photosynthesis (Wells 1991). Therefore, the degree of interference is not a monotonic function with respect to inter-plant spacing (Franco and Harper 1988).

Leaf area development involves the production of new leaves, increase in the size of existing leaves and the senescence of old leaves. It is a fundamental process of crop production as leaves intercept solar radiation and produce carbohydrates through photosynthesis. The production, expansion and survival of green leaf area are important determinants of crop productivity, as are the relative durations of the vegetative and reproductive phases (Ranganathan *et al.* 2001).

Table 5.3. A summary of the ANOVA for different variables and the factors affecting growth of *Brassica juncea* var. Ensabi.

		Mean squares									
Source of variation	D.F	Plant height (cm)	Total leaves / plant	Total pods / plant	Seeds / pod	Pod length (cm)	Stem diameter (mm)	Biomass (g)	Weight of leaves (g)	Weight of stem (g)	Leaf / stem
Replication	3	6.89	3.06	0.60	0.89	0.041	0.18	0.01	0.001	0.014	0.001
Space	6	2422.83**	26.74**	15.36**	26.74**	0.91**	7.31**	4.44**	4.08**	17.79**	0.01**
Error	18	4.09	4.79	2.73	0.20	0.01	0.09	0.001	0.001	0.008	0.001
CV		12.20	12.31	15.83	5.18	12.97	16.55	12.10	11.96	12.87	15.89

** Significant F-test at $p< 0.01$.

Table 5.4. Effect of growing space on the different growth parameters of *Brassica juncea* var. Ensabi.

Growing space	Plant height (cm)	Total leaves / plant	Total seeds / pod	Pod length (cm)	Stem diameter (mm)	Biomass (g)	Weight of leaves (g)	Weight of stem (g)	Leaf / stem
1	60.25 f*	59.25 f	5.00 d	1.88 e	2.88 d	0.71 f	0.32 f	0.29 f	0.55 a
2	64.00 f	63.00 f	5.75 d	2.03 e	3.25 d	0.77 f	0.37 f	0.40 f	0.47 b
3	78.75 fe	72.00 e	7.50 c	2.35 d	4.10 c	1.31 e	0.62 e	0.69 e	0.45 bc
4	86.25 d	87.25 d	9.25 b	2.65 c	4.66 bc	1.96 d	0.84 d	1.03 d	0.42 cd
5	94.25 c	102.30 c	10.00 b	2.85 b	5.18 b	4.05 c	1.97 c	2.35 c	0.43 c
6	111.25 b	124.30 b	11.25 a	3.05 a	5.98 a	9.08 b	4.07 b	5.09 b	0.40 d
7	129.50 a	156.30 a	11.50 a	3.05 a	6.53 a	12.42 a	5.63 a	6.84 a	0.39 d
S_x	1.01	1.09	0.22	0.04	0.15	0.06	0.016	0.045	0.05

* Values followed by similar letters within the same column are not significantly different at $p < 0.05$.

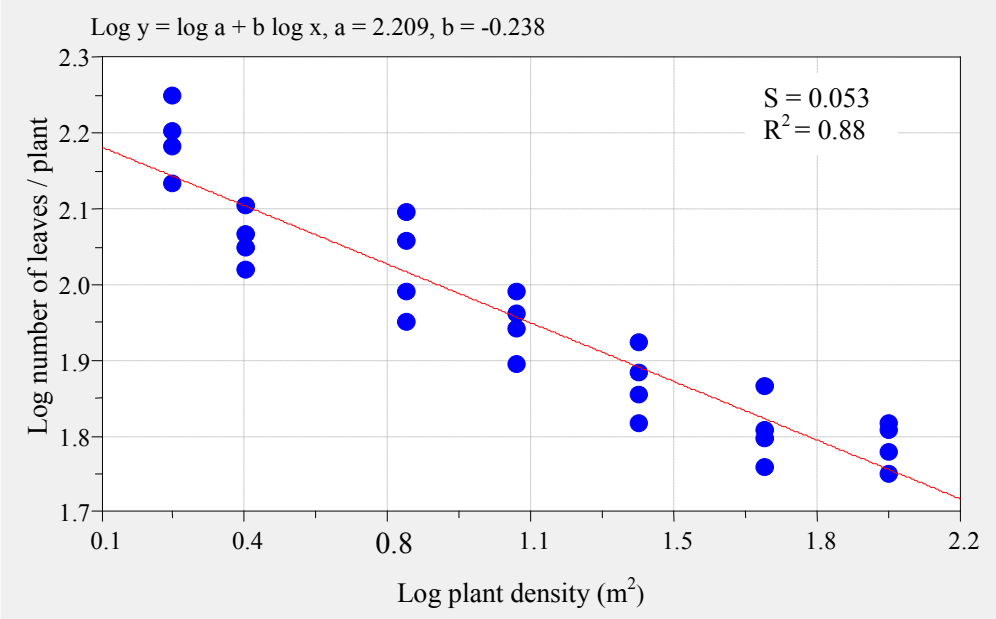

Fig. 5.6. Linear logarithmic model for number of leaves per plant of *Brassica juncea* var. Ensabi as a function of plant density (m^2).

The number of pods per plant, number of seeds per pod, and the pod length of *B. juncea* in different plant densities are depicted in **Tables 5.3** and **5.4**. The HSD tests showed that progressive increase in the number of pod per plant, seed per pod and length of pods in the low-density regime populations (2-7 plants/m^2) and growing space significantly influenced them.

The results revealed the number of pods per plant increased with decreasing plant population in all growing spaces (**Figs. 5.8** and **5.9**). Arc 1 recorded the higher pod per plant and showed significantly (p<0.01) differences to compare with the other arcs. There were no significant (p<0.05) difference in the number of pods per plants or along arcs 5-6-7. Angadi et al. (2003) in Canada documented that under the favorable conditions very low populations of canola (5 plants m^2) produced six times more pods per plant than high populations (80 plants m^2). At lower plant populations, between plant competition is reduced. Individual plants then grow larger, have bigger stems, branch more profusely and produce more pods that generally extend lower on the plant.

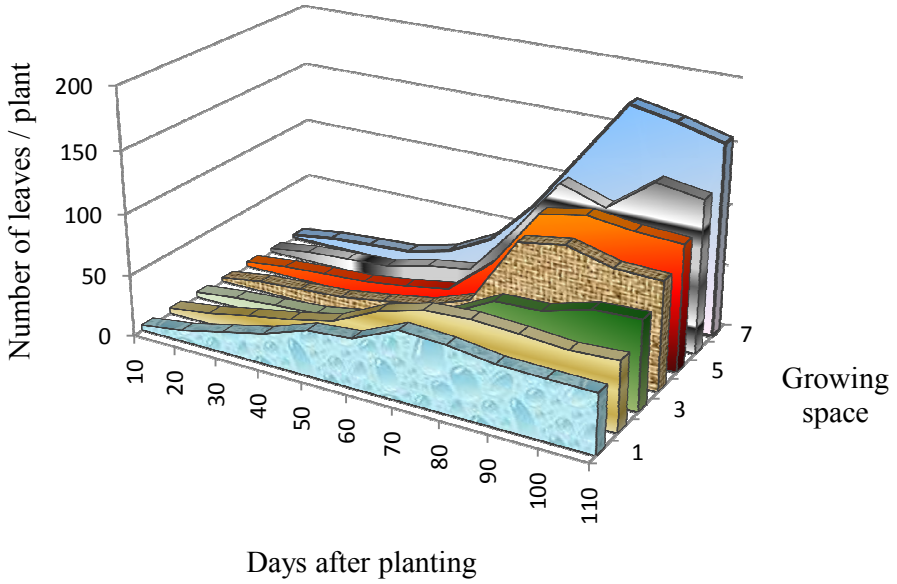

Fig. 5.7. Effect of growing space on the number of leaves in *Brassica juncea* var. Ensabi for a period of 110 days after planting date (DAP) in a fan design experiment. 1, 2, 3….7 – arcs with different spatial arrangements.

The number of pods per plant is the most responsive of all the yield components in canola (Diepenbrock 2000) and is determined by the survival of branches, buds, flowers and young pods rather than the potential number of flowers and pods (Mc Gregor 1987). General observations regarding pod per plants in our study noted decreased rate of interference zones of competing increased number of pod per plant and increased pod number partially compensated for the decreased population. Similar partial compensation of pods were also observed by Morrison *et al.* (1990), Angadi *et al*. (2003) and Leach *et al.* (1999) and Yazdifar and Ramea (2009) reported a decrease in number of pods per plant due to an increase in plant density.

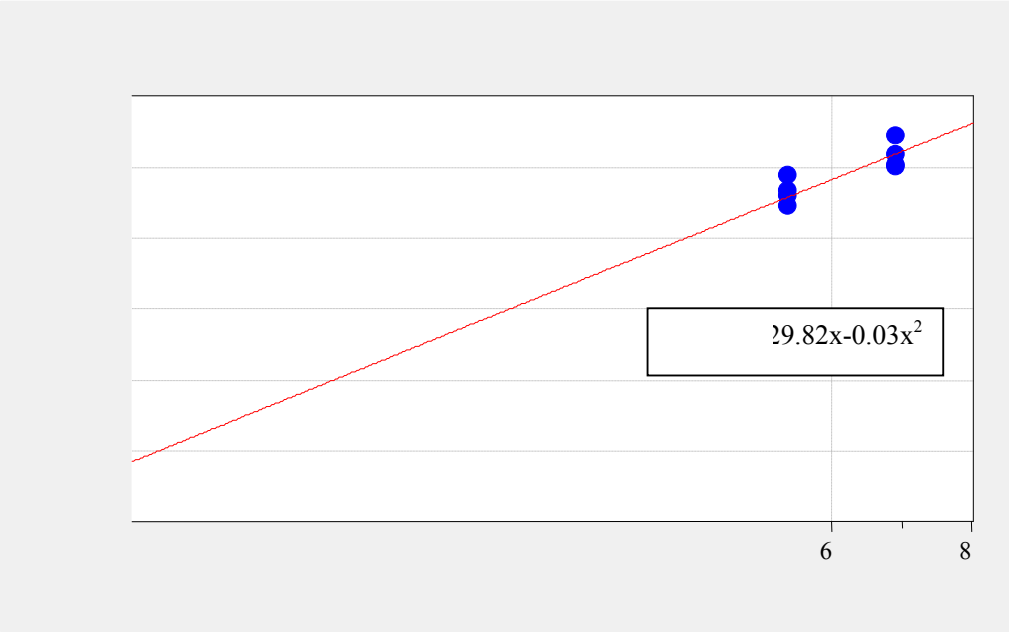

Fig. 5.8. Influence of growing space of *Brassica juncea* var. Ensabi on total number of pod per plant at harvesting time (110 days after planting) in a fan design experiment. 1, 2, 3….7- arcs with different spatial arrangements.

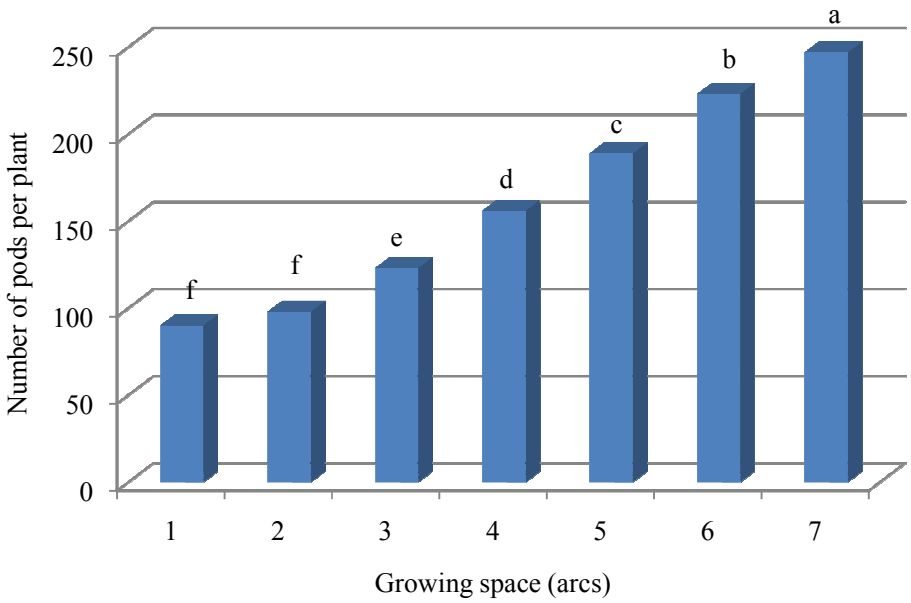

Fig. 5.9. Total pods per plant in *Brassica juncea* var. Ensabi at maturity as a function of different growing spaces in a fan design experiment. 1, 2, 3….7 – arcs with different spatial arrangements. Figures followed by the same lower case letters are not significantly different (Tukey's HSD Post Hoc Test) (p<0.05).

As plant density increases, each plant produces less dry weight, thinner stems, fewer branches, fewer pods and fewer seeds per plant due to increased competition from adjacent plants. However, fewer seeds per plant are offset by a higher number of plants, resulting in a similar seed yield per unit area compared with lower plant populations.

Table 5.5. The regression table for different measured characters (y) of *Brassica juncea* var. Ensabi in growing space (x) for linear logarithmic model (LLM): Log y = log a+ b log x and linear regression model (L): y=a+bx.

		Mean squares		
Source	Df	Number of pod / plant	Number of seed / pod	Pod length
Regression model	1	0.69** L	0.68** L	0.29** L
Residual	26	0.005	0.002	0.01
a		2.47	1.23	0.56
b		-0.27	-0.28	-0.28
t- value for r^2		-7.43**	-17.14**	-14.51**

** F test significant at p <0.01, a (constant amount) and b (coefficient of the variable).

The results of the growth, yield components and yields of *B. juncea* var. Ensabi are as influenced by different growing spaces, are summarized in **Tables 5.3** and **5.4**. The Tukey's Honestly Significant Difference Test (HSD) showed that the significant (p < 0.01) influence of growing space on number of seeds per pod. Further, the results indicated by increasing density, the number of seed per pod decreased where there was no statistically differences between arcs 1-2, 4-5 and 6-7 (**Fig. 5.10**). The increase in density, has led to parallel increase in inter- plant competition, and plant ability for utilizing environmental resources and consequently plant dry matters likewise dwindle. To resolve the stress and to establish equilibrium among

photosynthesis, respiration and storing nutritional matters, the plant hastens filing out the grains resulting in a decrease in the number of branches, number of pods per plant, number of seeds per pod, the time of filling out seeds and 1000-seed weight. Seeds number per pod in plants is a function of resource drawing ability of ovules (Angadi *et al*. 2003).

This is agreement with previous studies by Morrison *et al.* (1990) who reported significant increase in seed per pod with reducing population and contrasts with the observation of Angadi *et al*. (2003) and Yazdifar and Ramea (2009) that they documented there was no significant differences in seed per pod were observed.

The availability of growing space significantly influenced pod length (**Table 5.4**). It follows that pod length were measurably longer in wider spacing than those at closer spacing. Although long pods generally produced greater weight of seeds per pod than short pods, the advantage in seed number and/or the weights of individual seeds was less than that expected pro rata for the much greater pod length. Indeed, and paradoxically, short pods produced more seeds per unit length of pod than long pods. This suggested a less efficient distribution of assimilates within the longer pods, a greater amount being required to support the growth of pod walls than in short pods. Alternative approaches to using pod length as a criterion of selection for high yield are discussed (Chay and Thurling 1989). Invariably, mean pod length per plant in arc 7 and 6 were statistically similar and the longest, then sharply dropped. As shown in **Fig. 5.11** the mean length of pod per plant lastly statistically similar in arcs 1and 2.

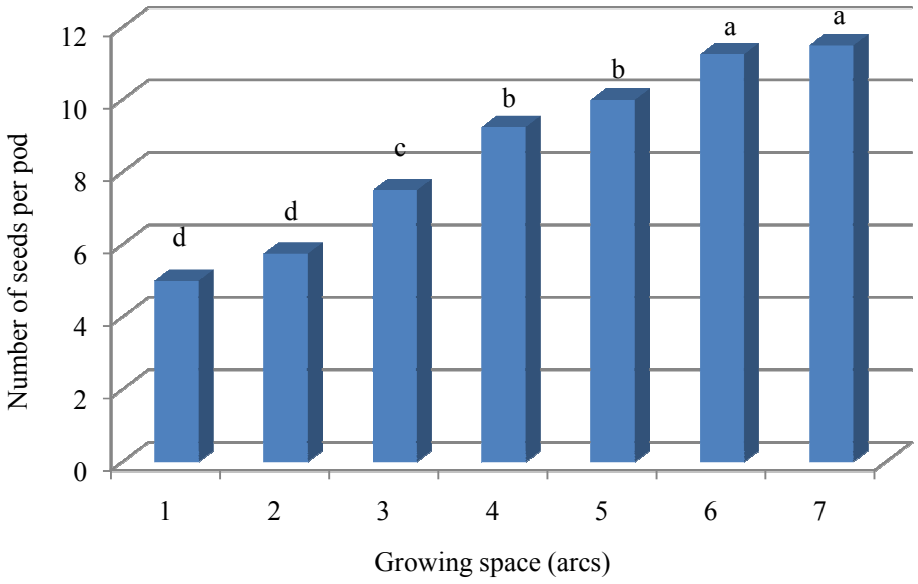

Fig. 5.10. Effect of growing space on the number of seeds per pod in *Brassica juncea* var. Ensabi at maturity in a fan design experiment. 1, 2, 3….7 – arcs with different spatial arrangements. Figures followed by the same letter are not significantly different (Tukey's HSD Post Hoc Test) (p<0.05).

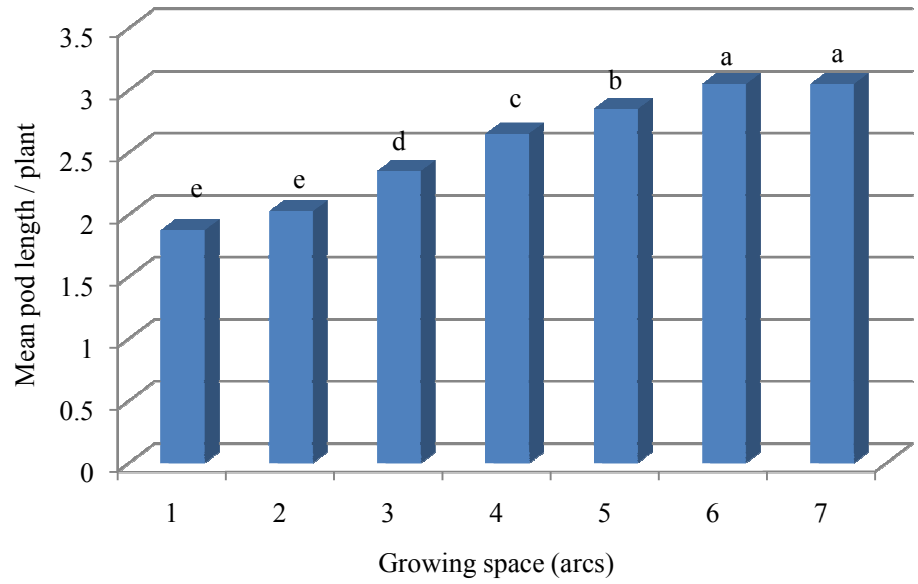

Fig. 5.11. Effect of growing space on the mean pod length in *Brassica juncea* var. Ensabi at maturity in a fan design experiment. 1, 2, 3….7- arcs with different spatial arrangements. Figures followed by the same letter are not significantly different (Tukey's HSD Post Hoc Test) (p<0.05).

5.3.3 Effect of growing space on final size of individual plants

The results of the plant sampling are summarized in **Tables 5.3** and **5.4**. In general, the plants became significantly ($p < 0.01$) larger and more robust with ensuing growth the branch length and stem diameter increased in bigger-spaced plants; and the leaves became larger. Increased number of the total pods per plant, number of seeds per pod and pod lenght were observed in this study. Similar results were reported with other plants (Franco and Harper 1988; Haruyuki and Kazuhiko 1999; Fravani 2009).

4.3.3.1. Effect of growing space on stem diameter

The regression analysis and one-way ANOVA indicate the significant ($p < 0.01$) influence of growing space on stem diameter of *B. juncea* var. Ensabi (**Table 5.3**). The relationship between *B. juncea* var. Ensabi's growing space and stem diameter exhibited strong linear logarithmic relation with $R^2 = 0.91$ (**Table 5.5** and **Fig. 5.12**). Results noted that there was a significant negative relationship between spacing and stem diameter, at the closer spacings were thicker than those at wider spacings. Mean comparison tests with HSD (**Table 5.4**) indicated that the differences in mean stem diameter at different growing spaces more (arcs 6-7) or less (arcs 1-2) than 7 plants/m^2 was not significant at $p < 0.05$. It also showed that was a high significant difference observed on the stem diameter of the *B. juncea* var. Ensabi in low and high plant density regimes.

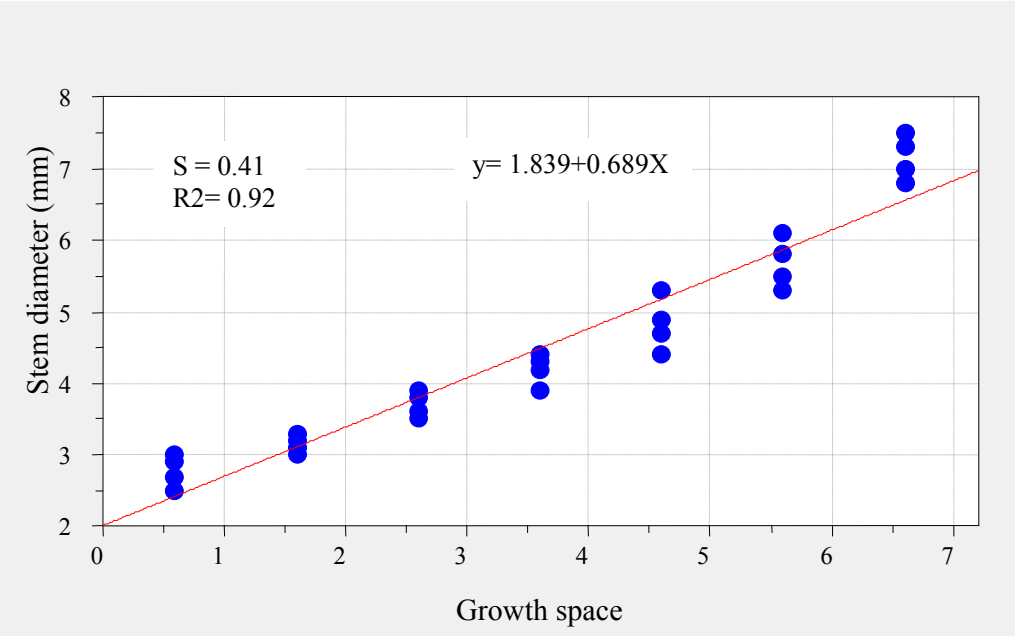

Fig. 5.12. Effect of growing space of *Brassica juncea* var. Ensabi on stem diameter at harvesting time (110 days after planting) in a fan design experiment.

Table 5.6. ANOVA table for regression in different measured characters (y) of *Brassica juncea* var. Ensabi in growing space (x) for linear logarithmic model (LLM): Log y = log a+ b log x and linear regression model (L): y=a+bx.

		Mean squares			
Source	Df	Stem diameter (mm)	Biomass / plant (g)	Weight of stem / plant (g)	Weight of leaves/ plant (g)
Regression	1	1.86**	97.84**	5.83**	97.27**
model		L	LLM	L	L
Residual	26	0.02	0.55	0.26	0.57
a		0.46	6.96	1.47	6.92
b		0.45	1.92	-0.01	6.19
t- value for r^2		27.79**	13.40**	-4.69**	13.10**

** F test significant at $p<0.01$, a (constant amount) and b (coefficient of the variable).

4.3.3.2. Effect of growing space on total biomass

Plant density has the greatest effect on biomass, yield and the yield components of individual plants. Biomass is an important parameter for the characterization of eco-systems since it reflects the ecosystem's capacity, during a certain time, in accumulating organic matter (Overman *et al.* 1994). When plants were grown over a range of inter–spaces, mean plant size (weight) decreased as spacing decreasing (**Fig. 5.13** and **Table 5.6).** This plasticity in plant growth can be formalized in the form of an empirical and precise relation (**Fig. 5.12**). Arguably; either the plants have not reach a final constant yield, or the plants have attained constant final yield, but this was obscured in the results by the way as we have defined "density" "as the inverse of the average available space per plant in each arc at the beginning of the experiment".

The Tukey's Honestly Significant Difference Test (HSD) revealed plant density significantly (p<0.01) influenced Total biomass of *B. Juncea* var. Ensabi. Accordingly, the total biomass per plant? in wider density significantly higher than those in closer density **(Fig. 5.13).** The 'densities' were calculated on the basis of the initial space potentially available to each plant in the design, and this did not provide enough information on the dynamic nature of space occupation.

Linear regression for biomass size shows y= 21.23X + 0.659 with coefficient determination R^2=0.96 of the relationship between biomass (y) and growing spaces (x). The best regression equations to estimate weight of stems and leaves were similar to that between total dry plant weight (biomass) and growing space (**Fig. 5.14**).

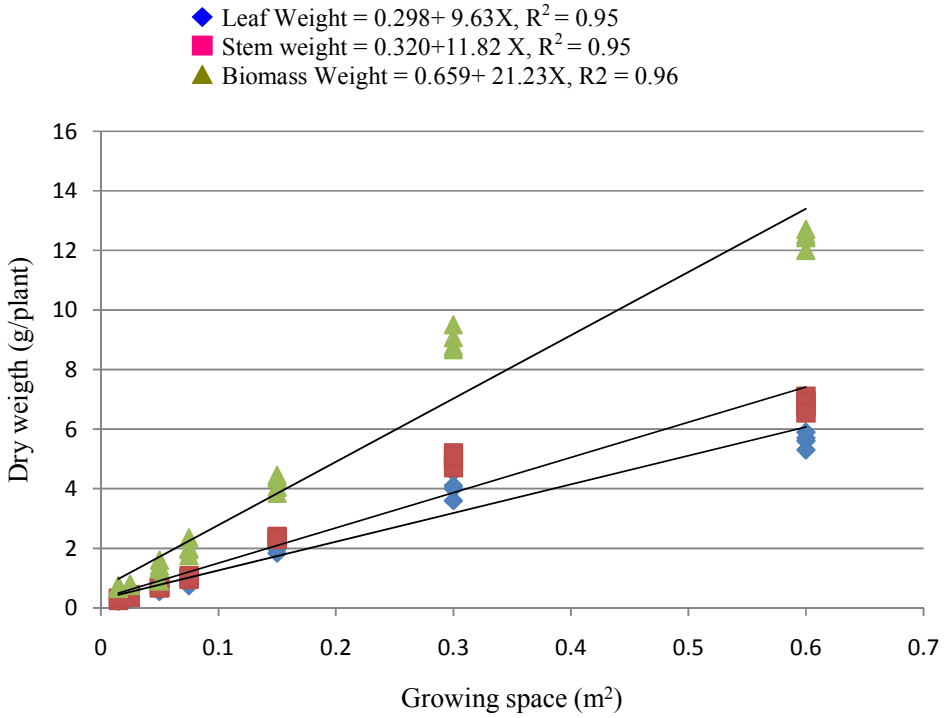

Fig. 5.13. Linear models showing the effect of growing space on *Brassica juncea* var. Ensabi as estimated by aerial biomass (g/plant).

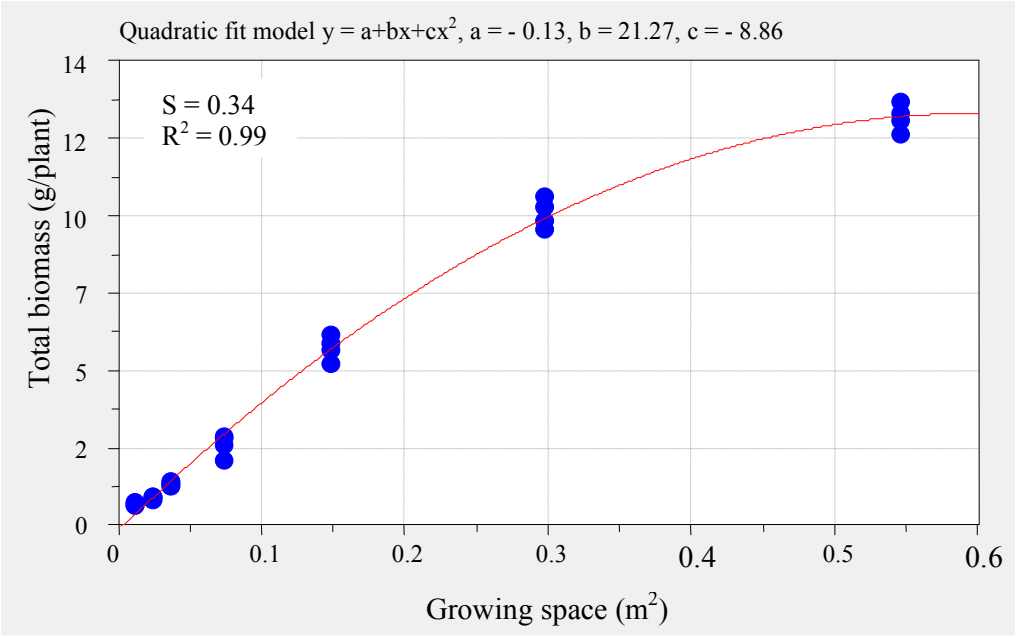

Fig. 5.14. Quadratic association model for the effect of growing space on aerial plant biomass (g/plant) in *Brassica juncea* var. Ensabi.

The growth patterns of *B. juncea* based on different growing spaces suggest that in spacing gradients 'density waves' are transmitted along the rays of the design and away from the origin of the polar co-ordinate. Given the fact that asymmetrical competition occurs when a plant dies, leaving a gap in the canopy into which already, existing neighbours or newly recruited plants can invade, or whenever an individual acquires an advantage over its neighbours (a richer patch of soil, an earlier germination, some resistance to pathogens or herbivores, etc.).

The plants of *B. juncea* var. Ensabi showed a very high survival percentage even when planted in high densities. Closely spaced plants were significantly taller than the more narrow-spaced plants of the same age. The total biomass, leaf weight, stem weight, plant height, number of total pod per plant, number of seed per pod and number of leaves were greater in wider spaced plants. These results implied that as the area occupied by each individual plant decreased, or in other words as the plant density increased, the rate of mortality increased as a result of self thinning that happened in two plants in arcs 1 and 2. The occurrence of self-thinning was probably due to the prevailing conditions such as low light quantum, competition for space and nutrients created by the neighbouring plants growing in a smaller area. Periodic observations indicated that plants in the outer circles tended to grow and lean in the direction of the inner circles, which might have caused the death of *B. juncea* var. Ensabi in the inner circles because of density-independent factors.

The results of this study demonstrated that the inner plants in the Nelder's fan design experiment could not use environmental resources, especially sunlight, properly. It could be argued that the timing of stem elongation in the brassica plants here strongly affected competitive success for plants to increase light interception for elongation at early life history stages (Weinig 2000). In addition, the study revealed that plant spacing, growth rate and harvest are interrelated critical factors in optimising biomass production. Higher density enhanced restraint of biomass accumulation.

Plant competition for light is a commonly occurring phenomenon in natural and agricultural vegetations. Under competitive conditions, stem elongation in plants is thought to enhance fitness by increasing light interception. Plants showed plastic growth responses to high plant densities as in this study. Plant height increased with decreasing plant density (Franco and Harper 1988). However, other studies reported that stem elongation did not prevail in transgenic plants; they cannot sense neighbours and, therefore, show no shade avoidance responses (Weinig 2000; Pierik *et al.* 2004). Further, the plant's ability to respond morphologically to the presence of neighbour plants with enhanced shoot elongation, the so-called shade avoidance response increased with increasing plant densities (Pierik *et al.* 2004). The fitness benefits of elongation may therefore depend on the timing of this plastic response. Due to the rapid growth rate and high density of plants in disturbed areas, selection to increase seedling-stage elongation may occur in crop plants in weedy sites. Individuals that elongate would experience the carbon cost of allocating to structural tissue, but fail to experience a carbon return through increased light interception.

CHAPTER 6

ASSESSMENT OF THE CHEMICAL NATURE AND ALLELOPATHIC POTENTIAL OF PLANT EXTRACTS FROM *BRASSICA JUNCEA* VAR. ENSABI

6.1 INTRODUCTION

The Malaysian flora is one of the richest and the oldest flora in the world. According to Network 2005, Malaysia is one of 12 centres of mega-biodiversity in the world and more than 15,000 species of flowering plants are known in this tropical climate (Network 2005).

Plants belonging to the *Brassica* oilseed crops have become the third most important source of edible vegetable oils in the world (Cartea 2008). These plants are grown not only for their oil, which is valued as both edible and industrial oil, but also for their meals, which is a good source of protein for both animal and human consumption (Uppstrom 1997; Gunasekera 2006). The air-dried seeds normally contain about 35- 44% oil (Downey 1993; Gunasekera 2006) and the meal of *Brassica* species is very rich in protein, about 36–44% (w/v) protein after oil extraction and 20–30% protein on a whole seed are available (Uppstrom 1997; Burton 1999; Gunasekera 2006).

Ensabi has been grown as a special crop in east part of Malaysia for many years but has not been used extensively for oil production although it contains a relatively higher oil content of its seed. Very little is known on the chemical composition of seeds of this plant.

While *Brassica* oils are low in aliphatic glucosinolates and erucic acid, the varieties are increasingly referred to as canola, a more pleasant-sounding name. Canola, which is most often *B. napus,* has received much attention worldwide and may soon be the most popular oilseed crop. Now canola-quality *B. rapa* and *B. juncea* varieties are also available (Sinha 2007).

Brassica oil production has been considered beneficial from the health point of view. It has linoleic acid, desirable for nutritional purposes and oleic acid, which being thermostable, is desirable as cooking oil. High oleic acid oil tastes better and may also has health benefits. The oxidative stability of this fatty acid makes it suitable for some industrial applications too. Nevertheless, Brassica oil is characterised by significant amount of erucic acid (about 50% of the total fatty acids) which is absent in any other commercial plant oil. Oil containing high erucic

acid content has anti-nutritional properties but is suitable for some industrial applications (Cartea 2008).

Indian mustard (*B. juncea* L. Czern.+ Coss.) is an important animal and human food source due to its high quality oil and high protein seed meal with a complete component of amino acids including lysine, methionine and cysteine. However, presence of high glucosinolate content in the seeds of some varieties, the meal of these unacceptable for animal and human consumption (Font 1999; Iqbal 2008).

6.1.1 The chemical nature of *B. juncea* var. Ensabi

6.1.1.1 Oils

Speciality oils having high content of a specific fatty acid are of immense importance for both nutritional and industrial purposes. Oil rich in oleic acid has high demand in commercial food-service applications due to its long shelf-life and cholesterol-reducing properties (Kaushik 2000).

Mustard oil from *B. juncea* is one of the primary cooking oils in South Asia, with large production in both India and Pakistan. By value of to canola oil quality, many nutritionists are interested in it. Fatty acid composition of canola oil is very different from the other edible vegetable oils (Downey 1990). Mustard seeds contain a large proportion of oil (27–36% by weight) with high erucic acid levels, very similar to traditional rapeseed. Canola-quality of Indian mustard (*Brassica juncea*) is being developed as a complimentary oilseed crop to canola (*Brassica napus*) for cultivation in hot areas, where canola does not perform well (Wijesundera 2008). The potential benefits of its quality are recognized by a number of northern hemisphere countries, particularly Canada, where there are major breeding programs focused on its development (Norton 2004; Wijesundera 2008).

6.1.1.2 Fatty acids

Nutritional value of different vegetable oils is dependent on the nature of different fatty acids present (Canadian Grain Commission 2008). Seed lipids of higher plants are mainly composed of C16 and C18 fatty acids. Palmitic, stearic, oleic, linoleic, linolenic, eicosenoic and erucic acids are the seven major fatty acids present in the oil extracted from members of the family Cruciferae (Sovero 1993; Mandal 2002).

Different species of Brassica including mustard such as *B. juncea*, *B. carinata*, *B. nigra* and *B. napus* contain long chain Fatty acids (LCFA) which have more than 18 carbons such as C20:1 (eicosenoic acid) or C22:1 (erucic acid) (Leonard 1994; Kanrar 2006). *B. juncea* contains lower amount of linoleic acid and higher levels of oleic and linolenic acids as compared to *B. napus* (Wijesundera 2008).

6.1.1.3 Proteins

Proteins form an important group of biological molecules, which function as enzymes, antibodies, cell structure components and storage molecules within any living organism. This range of functions can be attributed to the variety of structures which different protein molecules can have, and the numerous interactions that are possible between sections of a protein molecule and between different molecules.

After the extraction of oil from seeds of oilseed crops, the residual mixture is a valuable source of high-proteins for commercial compound feeds. The relatively high protein content (18–24%) of rapeseed and mustard varieties make them suitable sources of food grade vegetable proteins. The balance of amino acids found within the seeds of these crops compares favourably with human nutrition (Marnoch 2006).

6.1.1.4 Glucosinolates

Glucosinolates are a group of nonvolatile nitrogen and sulphur-containing secondary plant metabolites that are thought to be involved in plant defence against insect herbivores,

pathogens, nematodes, and other competing plants, synthesized by thousands of plant species including the agriculturally important *Brassicaceae* (Fahey *et al.* 2001). At least 120 different glucosinolates have been identified in these plants (Fahey *et al.* 2001; Hopkins 2009).

Numerous reviews have addressed the occurrence of glucosinolates in vegetables, primarily the family *Brassicaceae* that are responsible for the desirable pungent odor and sharp flavor associated with these foodstuffs (Canadian Grain Ccommision 2008). These glucosinolates and their breakdown products contribute to plant defence, human and livestock health, and the sensory quality of vegetables (Rosa 1998).

Distribution of the glucosinolate levels is highly dependent on planting date (Ciska 2000), plant organs and growth stages between seed planted and seed harvested, with both quantitative and qualitative differences between roots, leaves, stems and seeds (Fahey *et al.* 2001; CGC 2008; Hopkins 2009).

6.1.2 Allelopathy

The current trend in agriculture production is to find out a biological solution to reduce the perceived hazardous impacts from herbicides and insecticides (Khanh *et al*. 2005). The allelopathic properties of plants may act as a biological weed control mechanism in the agro-ecosystems and are effective tool to help resolving this critical issue (Xuan *et al*. 2005).

Plant compounds showing the effect of suppressing or poisoning neighboring plants upon their release into the environment are known as allelochemicals and the phenomenon is called allelopathy. Allelopathy is generally associated with interactions between living plants and has been observed in agricultural lands for centuries. The term 'allelopathy' was introduced by Molisch (1937) to refer to biochemical interactions between all types of plants, including microorganisms. Molisch meant the term to cover both inhibitory and stimulatory biochemical interactions.

Allelopathy plays a significant role in plant-plant interactions. Growing environmental and public health concerns from the use of agricultural chemicals (pesticides) in agriculture has stimulated interest in the search for new and environmentally safe technologies. The ability of some natural plant compounds to effectively inhibit weeds has opened new horizons for future research (Rice 1984).

Allelopathy is defined as a natural phenomenon that involves the direct or indirect harmful or beneficial effects of one plant on another through the production of chemical compounds that escape into the environment (Brown 1991; Turk 2005). It is an interference mechanism by which plants release chemicals which affect other plants; and it has often been proposed as a mechanism for influencing plant populations and communities (Khanh 2007). Rice (1984) defined allelopathy as an important phenomenon observed in many plants that release chemicals into the neighbouring environment either from their aerial or underground parts in the form of root exudation, leaching by dews and rains, and volatilization or decaying plant tissues (quoted by Fujii 2003). Another definition of allelopathy is an interaction among plants by chemical pathways and this interaction includes both inhibition and promotion (Khanh *et al*. 2005). Allelochemical-based herbicides are natural products and thus could be broken down easily by microorganisms, making them less persistent in the environment (Chon 2002; Singh 2003; Xuan 2005; Khanh 2007).

Increasing attention has been given to the role and potential of allelopathy as a management strategy for crop protection against weeds and other pests. Incorporating allelopathy into natural and agricultural management systems may reduce the use of herbicides, insecticides, and other pesticides, reducing environmental soil pollution and diminish autotoxicity hazards. There is a great demand for compounds with selective toxicity that can be readily degraded by either the plant or by the soil microorganisms which provide new strategies for maintaining and increasing agricultural production in the future (Inderjit and Mukerji 2006; Faravani *et al* 2008).

Allelochemicals are released from the plants through leachation, volatilization, root exudation and the death and decay of the fallen plant parts either through biotic or abiotic means. Upon release, these are involved in a number of metabolic and physico-chemical processes (Rice 1984; Einhellig 1996). Crop allelopathy may be useful to minimize serious problems in the present agricultural production such as environmental pollution, unsafe products, human health concerns, depletion of crop diversity, soil sickness and reduction of crop productivity (Khanh 2005).

Research has shown that allelopathtic effect can reduce seed germination and seedling growth. Like synthetic herbicides, there is no common mode of action or physiological target site for all allelochemicals. Allelochemical concentrations in the producer plant may also vary with time and the plant tissue. For example, foliar and leaf litter leachates of *Eucalyptus* species are more toxic than bark leachates to some food crops (Iqbal 2005). Several examples have shown the potential of allelochemicals as herbicides and these phytotoxic compounds from plants are used in the production of new herbicides. Phytotoxic compounds from plants and microorganisms represent a wide range of chemistry and mechanism of action that have potential in the design and development of new herbicides (Duke 1987; Khalid 2002).

Several Brassica species are reported to have a phytotoxic potential (Tawaha and Turk 2003). When they are grown in crop rotation, may have allelopathic effects on other plant species, and reduce seed germination and suppress weeds (Bialy 1990; Turk 2005). Some plants from this family such as mustard (*B. juncea*) have a high potential to be used in alternative weed management. Mustards (*B. juncea*) have been genetically bred for increased glucosinolate content in roots and shoots. The glucosinolates that are produced in *B. juncea*, show allelopathic effect such as; can inhibit germination, suppress plant growth and control weeds as herbicides if weed seeds were targeted (Brown 1995). The "Sandwich Method" enables to assay the allelopathic activity of the leaf litter of a donor plant inserted in agar medium, and to evaluate the

effect on the radicle growth of a receptor plant (Fujii 2003). Little is known about allelopathy of *B. juncea* var. Ensabi.

6.2 MATERIALS AND METHODS

6.2.1 Oil content

Oil content can be defined as the maximum amount of lipid material that can be removed from the seed by extraction with specific solvents (usually hexane or petroleum ether). The oil content was detected using the mixed sample of seeds by the soxhlet extraction method using hexane.

Brassica juncea var. Ensabi seeds were obtained from the mature plants of a field of experimental pots from RIMBA ILMU at University of Malaya. Harvested seeds were dried in an air-circulating oven at 45°C for 24 hours. Five grams of the dried seeds were grounded with a laboratory mill (Hosokawa Alpine 100 UPZ), kept in the soxhlet apparatus and 250 mL of hexane (boiling point 65-70°C) was added. Oil was extracted from the dried milled seeds for 10 hours. The solvent was recovered and distilled off at 70°C and the oil recovered in minimum volume of hexane was transferred into a pre-weighed clean beaker. The solvent was evaporated at 55°C in incubator and the oil was weighed. Oil content was calculated from the weights of oil and seeds using the formula;

$$\text{Oil content (\%)} = \frac{oil\ weight}{seed\ weight} \times 100.$$

Four replications were made.

6.2.2 Fatty acid composition

The determination of fatty acid composition was carried out by extraction of a portion of the oil and its chemical conversion into individual fatty acids. The fatty acids were then converted to methyl esters, compounds which can easily be converted to gases. The different fatty acids were then separated and analyzed by gas chromatography (GC), which is a more

convenient and precise method for qualitative and quantitative analysis of fatty acids (Kowalski 2007).

Chromatography was performed with Unicam 610 Series Gas Chromatograph equipped with a flame-ionization detector and a 120 m × 0.25 mm i.d. column coated with a 0.25 μm film of HP-6890. Split injection (split ratio 1:100) was performed, with hydrogen as a carrier gas at a flow rate of 43 m s^{-1}. The column temperature was maintained at 198°C for 1 min after injection then programmed at 2.75° min^{-1} to 225°C, which was held for 2 min, followed by at 40° min^{-1} to 230°C, for 2 min. The injection port and detector temperatures were set at 250°C. Calculations were based on analysis of standard mixtures and calculation of individual correlation coefficients.

6.2.3 Protein content

The analysis of protein content was based on the estimation of nitrogen content in the seeds. As protein is the major nitrogen containing component in seeds, its content was estimated by multiplying the nitrogen content by a factor (by convention, 6.25 for oilseeds).

Kjeldahl method was used for the quantitative estimation of protein in the seeds of *B. juncea* var. Ensabi using the method of Harbome (1973). The grounded, dried material was digested in concentrated sulfuric acid for converting all the nitrogen to ammonia. The ammonia was distilled into a standardized acid solution and determined analytically by titration (Appendix 5).

6.2.4 Electrophoretic characterization

Plant materials

Mature seeds of *B. juncea* var. Ensabi collected from Ensabi plants in the University of Malaya campus, were stored with silica gel in darkness at 4 ^{o}C before use. Fifty fresh seeds were placed in each petri-dish, previously lined with 9-cm diameter Whatman No.1 filter paper. The filter paper was moistened with 6 mL of water. The petri-dishes were placed in growth chambers

with temperature regime of 25 ^{o}C, and exposed to fluorescent light with intensity of 630 $Em^{-2}s^{-1}$. All petri-dishes were augmented with 6 mL of de-ionized water at 3-day intervals in order to maintain moisture conditions. Germinated seeds (complete seedlings) were removed from the petri dishes and used in subsequent experiment.

A part of seeds of Ensabi, were sown in wooden boxes previously filled with garden soil of Malacca series in an insect-proof house. The plants were subjected to 12 hours of natural sunlight outdoor (mean midday radiation of 1812 μmole photon m^{-2} s^{-1}), and 384 μmole photon m^{-2} s^{-1} inside in insect-proof house, and mean ambient temperatures of 33±2 ^{o}C (day) and 25±2 ^{o}C (night) at Rimba Ilmu, University of Malaya, Kuala Lumpur (3^{o} 8' N; 101^{o} 42' E), Malaysia. Plants were harvested at two different growth stages (start of flowering and physiological maturity stages). These plants were immediately washed with double distilled water to remove any soil or other adhered material and separated into shoots and roots.

Five grams of fresh seeds, whole seedlings, roots of seedlings, shoots of seedlings, fresh shoots and roots from each harvest were prepared and soaked separately in the buffer with pH 6.8 overnight. The samples were grounded with a mortar and pestle, mixed with 10 mL buffer, filtered through four layers of cheesecloth to remove the fiber debris, and centrifuged at 5500 rpm for 30 min at 5^{o}C. The supernatant was vacuum filtered again through Whatman No. 42 filter paper.

Determination of protein concentration

Protein concentration in various plant tissues was determind by Bradford's method (1976).

Bradford reagent: 50.0 mg of Coomassie Brilliant Blue G-250 dye (CBBG) was dissolved in 50 mL of 88% (v/v) phosphoric acid solution and 25.0 mL absolute ethanol (95%) and stirred overnight. The solution was made up to 500 mL with water and stirred for 30 min at

room temperature on a magnetic stirrer. The solution was then filtered through Whatman No. 1 filter paper and stored in a dark bottle at 4°C.

Standard protein solution: 5.0 mg bovine serum albumin (BSA) was dissolved in 25 mL of 0.06 M sodium phosphate buffer, pH 7.0 in a 25 mL standard flask.

Procedure

Constructed a standard curve by taking increasing volumes (0.1-1.0 mL) of standard BSA solution (5 mg/25 mL) in different tubes and the final volume in each tube was made to 1.0 mL with 0.06 M sodium phosphate buffer, pH 7.0. Protein samples were made in 0.25 mL of 1N NaOH and gently vortexed. Volume was made to 1.0 mL with 0.06 M sodium phosphate buffer, pH 7.0. Five mL of Bradford reagent was then added. The tubes were sealeded and gently vortexed to ensure proper mixing of the dye reagent. The reaction was allowed to continue for 5 minutes before absorbance values were read at 595 nm (**Table 6.1**). Protein concentration was determined from the equation generated from the standard curve (**Fig. 6.1**).

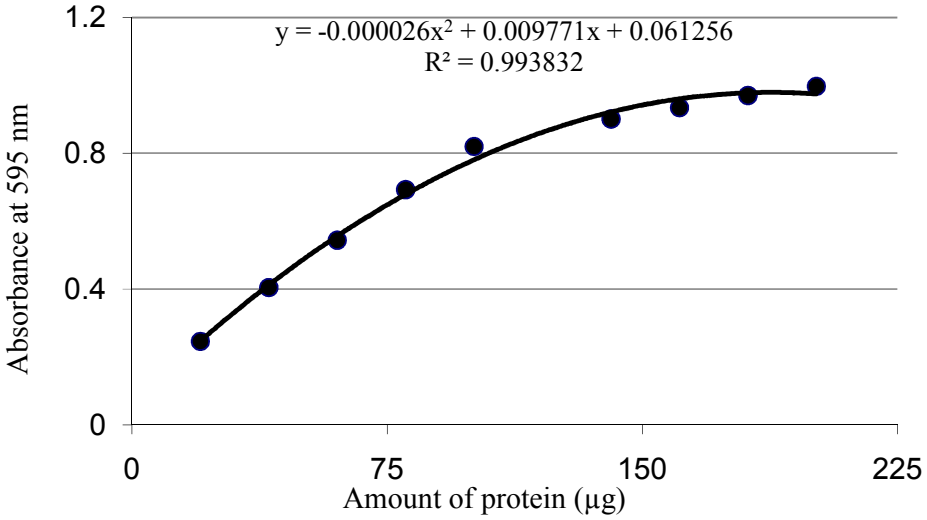

Fig. 6.1. Standard curve for the determination of protein concentration by the method of Bradford (1976).

Table 6.1. Amount of protein and corresponding absorbance values at 595 nm as determined by Bradford (1976) assay.

Tube No.	Amount of protein (µg)	Absorbance at 595 nm
1	20.12	0.25
2	40.24	0.41
3	60.36	0.54
4	80.48	0.69
5	100.60	0.82
6	140.84	0.90
7	160.96	0.93
8	181.08	0.97
9	201.20	1.00

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Several systems for SDS-PAGE have been described. In this experiment SDS-PAGE was performed according to Laemmli (1970) to study the protein profiles of different developmental stages of *B. juncea* var. Ensabi. Preparation of separating and stacking gel involves mixing of solutions A, B, C and D which were prepared in the following way.

Solution A [*29.2% (w/v) acrylamide and 0.8% (w/v) N, N-methylene bisacrylamide*]: 29.2 g of acrylamide and 0.8 g of N, N-methylene bisacrylamide were dissolved in water in 100 mL standard flask and stored at 4^{o}C in a dark bottle.

Solution B [*1.5 M tris-HCl buffer, pH 8.8 containing 0.4% (w/v) SDS*]: 18.5 g of tris and 0.4 g of SDS were dissolved in 80 mL of water. The pH of the solution was adjusted with 6 N HCl to 8.8 and the final volume was made up to 100 mL with water.

Solution C [*10% (w/v) ammonium persulphate*]: 0.02 g of ammonium persulphate was dissolved in 0.20 mL of water. The solution was prepared fresh.

Solution D [*0.5 M tris-HCl buffer, pH 6.8 containing 0.4% (w/v) SDS*]: 6.05 g of tris and 0.4 g of SDS were dissolved in 80 mL of water. The pH of the solution was adjusted with 6 N HCl to 6.8 and the final volume was made up to 100 mL with water.

Separating gel: It was prepared by mixing 3.0 mL of solution A, 3.0 mL of solution B, 2.945 mL of water and 0.05 µL of TEMED solution.

Stacking gel: It was prepared by mixing 0.28 mL of solution A, 0.5 mL of solution B, 1.278 mL of water and 0.04 µL of TEMED solution.

Electrophoresis buffer (0.025 M tris, 0.192 M glycine, pH 8.3 containing 0.1% SDS): 3.03 g of tris, 14.4 g of glycine and 1 g SDS were dissolved in 900 mL of water. The final volume of the solution was made up to 1000 mL with water.

Sample incubation buffer (62 mM tris-HCl buffer, pH 6.8 containing 10% glycerol, 2.3% SDS and 5% 2-mercaptoethanol): 0.75 g tris and 2.3 g SDS were dissolved in 80 mL of water with addition of 10 mL of glycerol, 5 µL of 2-mercaptoethanol and 0.001% of bromophenol blue. The pH of the solution was adjusted with 6 N HCl to 6.8 and the final volume was made up to 100 mL with water.

Fixing solution [*40% (v/v) methanol and 10% (v/v) acetic acid*]: 40 mL of ethanol and 10 mL of acetic acid were mixed and the final volume was made up to 100 mL with water.

Staining solution [*0.2% (w/v) coomassie brilliant blue R-250 in fixing solution*]: 0.2 g of coomassie brilliant blue R-250 was dissolved in the fixing solution and the final volume was made up to 100 mL with water. The solution can be reused 8-10 times.

Destaining solution [*5% (v/v) methanol and 7% (v/v) acetic acid*]: 5.0 mL of methanol and 7.0 mL of acetic acid were mixed and the final volume was made up to 100 mL with water.

Procedure

Gel wrap gasket set-up was prepared for gel casting using vertical mini-gel system (C. B. S. Scientific Co.) and checked for any leakage. Separating gel solution was prepared first as described above and immediately poured into the space between the two slab glass plates. Small amount of water was layered on to the top of the gel solution and the gel was allowed to polymerize for about one hour. After polymerization of the separating gel, stacking gel solution was prepared in the same way as described above and poured above the separating gel after removing the water layer. A gel comb was immediately inserted into the stacking gel to mark wells. After polymerization, the comb was removed and the wells were rinsed with electrophoresis buffer 3 times. The glass plates with the polymerized gel were fixed into electrophoresis unit and the tank was filled with electrophoresis buffer. Samples containing about 5-7 µg protein in 12 µL of sample incubation buffer were prepared. The samples were heated for 3-5 minutes at 95^{o}C and cooled down before loading. About 10 µL of each samples was loaded

in each well. The electrophoresis was allowed to run for about 2 hours until bromophenol blue front had reached to the bottom of the gel, first with 60 V and continued with 100 V when the sample had passed across the stacking gel. The gel was kept in fixing solution for 20 minutes, stained using staining solution for 15 minutes and destained using destaining solution by repeated washing at 37^{o}C on shaker.

The distance travelled by protein and bromophenol blue front was measured to calculate the relative mobility of each protein sample according to the formula given below;

Relative mobility = $\frac{\boldsymbol{Distance\ travelled\ by\ protein\ band\ (cm)}}{\boldsymbol{Distance\ travelled\ by\ bromophenol\ blue\ front\ (cm)}}$.

6.2.5 Glucosinolate content

Glucosinolates (GSL) are ionic or charged molecules. These have been analyzed using both wet chemistry and instrumental methods including paper, thin layer, gas and high performance liquid chromatography. Although many methods are available, there are advantages and disadvantages to each of them.

Glucosinolates were determined by first extracting them from the seeds into water followed by their isolation from interfering components. They were then converted into an uncharged molecule with an enzyme, and analyzed by UV vis spectroscopy. The current definition of canola requires that only part of the total glucosinolates in a sample (the aliphatic GSL) be measured.

Total glucosinolate measurement was performed according to enzymatically released glucose (Smith and Dacombe 1987). The method (Appendix) was based on glucose estimation after endogenous myrosinase hydrolysis of the intact glucosinolates. Extracts were filtered through charcoal-coated paper to remove interfering phenolic substances and glucose was measured by a colorimetric glucose oxidase/peroxidase procedure. Inactivation of myrosinase with acidified methanol/water mixture allowed free glucose in the seed to be measured

separately. Correction for free glucose was made before calculating the total glucosinolate content as μmol g^{-1}seed.

6.2.6 Allelopathy

Laboratory studies were conducted to assess the allelopathic potential of *B. Juncea* var. Ensabi as a natural herbicide on radish (*Raphanus sativus* L.) and barnyard grass (*Echinochloa crus-galli* [L.] Beauv.) respectively as indicator plant and weed species to see if *B. juncea* var. Ensabi could exhibit any differences in the inhibition of barnyard grass growth and development.

6.2.5.1 Aqueous extraction

Plant sampling and preparation of extracts: Plants were harvested at a vegetative stage from a field of experimental plots at University of Malaya and immediately washed with tap water to remove any soil or other adhered material and separated into leaves, stems and roots.

Fresh materials: Twenty grams of fresh materials (leaves, stems and roots) were chopped into small pieces (1–2 cm size, grounded and extracted with 100 ml distilled water at 25°C for 24 hours in a shaker. The extract was filtered through four layers of cheesecloth to remove the fiber debris and centrifuged at 3000 rpm for 4 hours. The supernatant was vacuum filtered again through Whatman No. 42 paper. Stock extracts were made fresh for each experiment.

Dried materials: Plant material was chopped into small pieces (1–2 cm size), dried in an oven at 40 °C for 48 hours, grounded into a fine powder using a Wiley mill to pass through a 1 mm screen and then stored in a refrigerator at 2°C in dark (Chung *et al.* 2001; Chon *et al.* 2005).

Twenty grams of dried leaves, stems and roots were soaked in 100 mL deionized water at 25°C for 24 hours in a shaker to get a concentration of 20 g dry tissue /100 ml (200g/ liter). The extract was filtered through four layers of cheesecloth to remove the fiber debris and

centrifuged on Eppendorf centrifuge, 5804 R at 10,000 rpm at 4 ℃ for 10 min. The supernatant was vacuum filtered again through Whatman No. 42 filter paper. Extracts were dried under vacuum. The plant powders were used in different bioassays.

Treatments:

- Control (C: sterile distilled water)
- Aqueous extracts: In screening experiments, the stock aqueous extracts from the fresh parts were applied as crude aqueous extracts at progressively increasing concentrations which were prepared using 0, 50, 100, 150, 200 and 300 gL^{-1} of the original dose.
- Stock extracts from dried powder method were diluted appropriately with sterile distilled water to give the final concentrations of 40, 80, 120, 160, 200 and 300 gL^{-1}.

Using above methods, solutions of similar concentrations were prepared separately from different parts of plants (roots, stems and leaves) and applied on the indicator plants and weed species. Thirty radish seeds were sown in 9 cm Petri-dishes lined with filter paper and 10 ml of each extract was applied. Treatment with distilled water was used as a control. The dishes were transferred to a growth chamber (set at 25 ℃, 4000 lux, time: 09:00–17:00 h).

Germination, shoot and root lengths and dry weights were determined after 7 days for all treatments. The inhibitory magnitude of each plant part was averaged from their inhibitory levels on germination, root length, plant height and dry weight of the indicator plant. The same method was used to study the effect of the aqueous extracts on the germination and early growth of selected weed species in a laboratory bioassay.

Weed seeds (30 seeds) were placed that 10 cm diameter petri dish lined with a Whatman filter circle that was moistened with 10 ml of respective aqueous extract or distilled water (to serve as control). Five replicates were maintained per concentration and plant part in a completely randomized manner in a growth chamber.

6.2.5.2 Sandwich method:

Leaf litters of *B. juncea* var. Ensabi were collected from the Rimba Ilmu, Institute of Biological Sciences, University of Malaya and these were subjected to analysis of their allelopathic effect using the sandwich method. Lettuce (*Lactuca sativa* L.) was used as a test plant material in the bioassay because of its reliability for germination and susceptibility to inhibitory and stimulatory chemicals (Fujii *et al*. 2003).

6.2.5.2.1 Preparation of leaf material

The experimental leaves (fresh) of Ensabi were collected, placed in a paper bag separately, then oven dried at 60° - 70°C for approximately 24 hours in an oven/drying chamber. After appropriate drying, the leaves were kept in a plastic bag and then in an airtight box until further use.

6.2.5.2.2 Preparation of growth medium

In order to avoid heat shock on the root, low temperature gelatinizing agar (Adar technical No. 3, OXOID, England) and gelatinizing temperature between 30° and 32°C were used. Agar concentration of 5% (w/v) was found best for the optimum growth of test plants and contained no added nutrients. The agar solution was cooled to 40°C before use.

Three replicates each of 10 and 50 mg oven dried leaves were placed in each well of six-well multidish plastic plate (10 cm^2 area for each dish) (Nalge Nunce Int., Denmark) for each sample. A total of 10 mL agar was layered in two layers (5+5 mL) on the dried leaves. Then five lettuce seeds were placed vertically, covered with plastic tape, labeled properly and incubated in dark for 3 days at 25°C (**Fig. 6.2**). Length of the radicle and hypocotyle was measured and the germination percentage of the seeds was determined (Fujii *et al.* 2003).

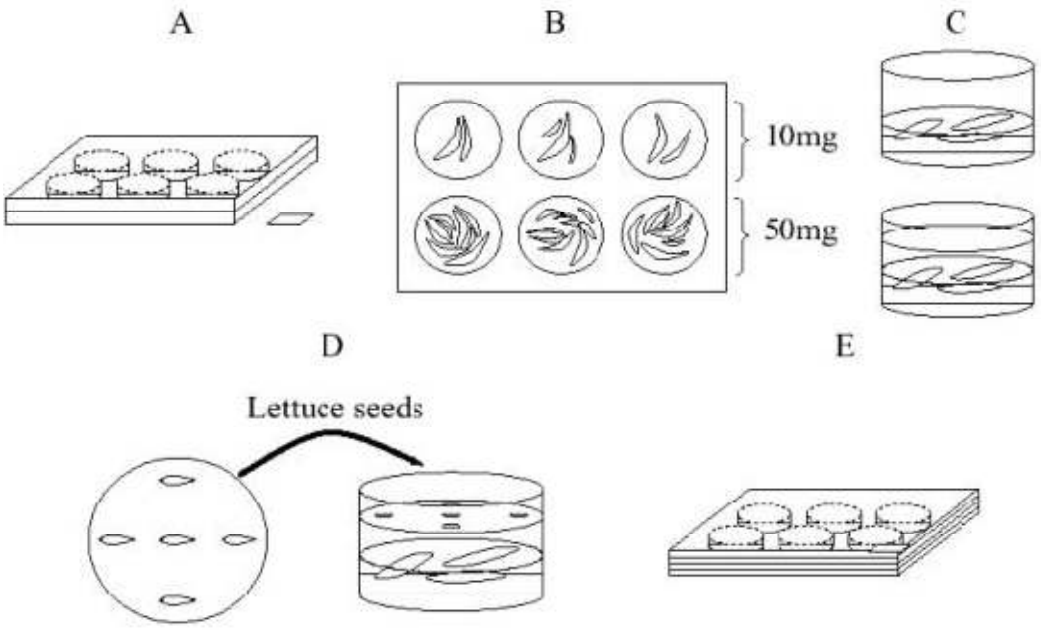

Fig. 6.2. Sandwich method: (a) six-well multidish plastic plate; (b) 10 or 50 mg dried leaves, stems and roots of *Brassica juncea* var. Ensabi placed in each well of the multidish plate; (c) addition of 5 mL plus 5 mL agar in two layers on the dried leaves; (d) five lettuce seeds placed vertically; (e) covered with plastic tape and appropriately labeled multidish incubated in the dark (Fujii *et al.* 2003).

6.2.5.3 Ethanolic extraction

Plants of *B. juncea* var. Ensabi were harvested at a vegetative stage from Rimba Ilmu, University of Malaya, Kuala Lumpur (3^{o} 8' N; 101^{o} 42' E), Malaysia and were immediately washed with tap water to remove soil or other adhered material. Then fresh plant parts (leaf, stem and root) of Ensabi were dried at room temperature. These parts were milled and homogenized. About 500 g of dried leaves, stems and roots of *B. juncea* var. Ensabi were subjected separately for extraction using ethanol as solvent in the Soxhlet extraction apparatus. The filtrate was concentrated under reduced pressure using the rotary evaporator (Dafaalla 2004; Aziz 2007).

6.2.7 Data analysis

Germination counts were performed for a period of 8 days for both radish and weed species, although calculations are based on the longest time taken to achieve maximum germination. The following parameters, previously reported by others (Jefferson and Pennacchio 2003) were calculated:

a) Final germination (FG) %: The maximum average percentage of seeds that germinated during the experiment.

b) Mean period of final germination (MPFG) = $(\sum_{i=1}^{d} NiDi)/FG$

c) Rate of germination (RG) = $\sum_{i=1}^{d} \frac{Ni}{Di}$

d) Percentage inhibition or stimulation=

$$\left(\frac{\text{FG in distilled water (\%)} - \text{FG in aqueous or ethanol extracts (\%)}}{\text{FG in distilled water (\%)}} \times 100\right)$$

Where d is the period of final germination; N_i is daily increase in seedling number; D_i is number of days from seed placement and the subscript i might be any integer value up through D.

Homogeneity of variances was tested and data showing deviation from normal destination were transformed, using $\arcsin\sqrt{x + 0.5}$; retransformed data were presented in the results. ANOVA was performed on the data. Differences between means were determined using Tukey's compromise test.

6.3 RESULTS AND DISCUSSION

6.3.1 Oil, oil components and other dependent compounds

Oil, protein and glucosinolate composition in different parts of *B. juncea* var. Ensabi is shown in **Table 6.2**.

Table 6.2. Oil, protein and glucosinolate composition in different parts of *Brassica juncea* var. Ensabi.

Component	Seed	Flower	Leaf	Pod	Stem	Root
Oil (%)	34.6	0.1	0.2	0.4	0.2	0.3
Protein (%)	32.1	0.4	1.3	0.5	1.1	1.1
Glucosinolate (µmol/g)	34.8	30.9	17.0	30.1	2.1	4.5

The average oil content of seeds was 34.6%, whereas other parts such as flower, leaf, pod, stem and roots contained less than 0.5% oil content. Seeds were also found rich in protein (32.1%) and glucosinolates (34.8 µmol/g). Other parts had around 1.3% protein. However, both flower and pod contained significant amount of glucosinolates.

The fatty acid composition of the Ensabi as determind by gas chromatography (**Fig**. **6.3**) is given in **Table 6.3**. The average oleic acid (C18:1) concentration in the oil from *B. juncea* var. Ensabi was 18.2% whereas the concentrations of other fatty acids were: linoleic acid (16.9%), linolenic acid (5.5 %), eicosenoic acid (8.8%), erucic acid (42.0%), stearic acid (1.4%), and palmitic acid (2.8%). All these fatty acids (except erucic acid) are important for dietary purposes. The high content of oleic acid further corroborates the possibility of using oil from Ensabi seeds in dietary formulations. Results showed the properties of fatty acids in Ensabi oil were comparable in quality to some other processed vegetable oils that are commercially available.

Fig. 6.3. GC analysis of fatty acids of *Brassica juncea* var. Ensabi.

Table 6.3. Fatty acid composition of oil of *Brassica juncea* var. Ensabi.

Fatty acid	Symbol	Amount (%)
Myristic	C14:0	0.1
Palmitic	C16:0	2.8
Palmitoleic	C16:1	0.3
Stearic	C18:0	1.4
Oleic	C18:1	18.2
Linoleic	C18:2	16.9
Linolenic	C18:3	5.5
Eicosenoic	C20:1	8.8
Erucic	C22:1	42.0
Unknowns		0.7

Oils with increased levels of oleic acid in combination with reduced linoleic and linolenic acid content show a higher oxidative stability, lower oxidation products and provide stability without extensive hydrogenation as the rate of oxidation of oils is influenced by the degree of unsaturation of fatty acids, light and temperature (Scarth and Mc Vetty 1999). In Canada where low glucosinolate cultivars were first introduced, the effect on the oilseed industry was so impressive that this new type of oilseed was designated as canola. By definition, canola is the seed of *B. Napus*, *B. juncea* or *B. Rapa*, whereby the oil component contains less than 2% eurcic acid (C 22:1) and the meal (solid) component possesses less than 30 μmol g^{-1} oil extracted

(Canola, 1990). According to this definition, the oil of Ensabi with very high content (42%) of erucic acid (C22:1) cannot be categorized as canola oil.

Table 6.4. Typical fatty acid compositions (in percentage) of the major commercial vegetable oils and their comparison with Ensabi (Smith 2007).

Fatty acid	Symbol	Rapeseed		Oil Palm		Soybean	Sunflower	Ensabi
		Napus (High erucic)	Canola	Mesocarp	Kernel			
Palmitic	C16:0	4.0	4.7	48.0	9.0	9.0	7.0	2.8
Stearic	C18:0	1.5	1.8	4.0	3.0	4.0	5.0	1.4
Oleic	C18:1	17.0	63.0	36.0	15.0	24.0	19.0	18.2
Linoleic	C18:2	13.0	20.0	10.0	8.0	54.0	68.0	16.9
Linolenic	C18:3	9.0	8.6			8.0	1.0	5.5
Eicosenoic	C20:1	14.5	1.9			0.0	0.0	8.8
Erucic	C22:1	43.0	0.0			0.0	0.0	42.0

The fatty acid compositions of *B. napus* and *B. juncea* oils are found different from those of other edible oils in possessing a substantial amount of the long-chain monoenoic (eicosenoic and erucic) fatty acids (**Table 6.4**). Vegetable oils are generally heterogeneous in their fatty acid composition. Although each of the fatty acids persent in oil has its own value, the most suitable oil must be homogeneous with respect to the presence of a very high percentage of a single fatty acid. Considerable progress has been made in recent years to increase the homogeneity of fatty acids that naturally exist in the major crop species (Smith 2007).

Table 6.5. Comparison of fatty acid profile of the seed-oil obtained from *Brassica juncea* var. Ensabi with that recommended by FAO/WHO for human nutrition[a].

	Average amount in seed-oil of Ensabi (*B. juncea*)	Recommended by FAO/WHO
Saturated fatty acids (SFA, C16:0 + C18:0)	Very low level (< 5%)	Low level
Monounsaturated fatty acid (MUFA, C18:1)	Moderate level (~18%)	High amount
Polyunsaturated fatty acids (PUFA, C18:2 + C18:3)	Moderate level (~22%) with a ratio (~3:1)	Moderate level with a desirable ratio (5:1 to 10:1)
Long-chain-unsaturated fatty acids (LCUFA, C22:1)	Very high content (42%)	Absence of LCUFA

[a]Report on 'Fats and oils in human nutrition' prepared by Food and Agriculture Organization of the United Nations and the World Health Organization, Rome, 19–26 October 1993 (Sinha *et al.* 2007).

The oil obtained from the Ensabi seeds showed low levels (<5%) of saturated (SFA) and moderate levels of monounsaturated (MUFA) (~18%) and polyunsaturated (PUFA) (22%) fatty acids (**Table 6.5**). Although present in small amounts, the two human essential PUFAs, *i.e.,* C18:2 (ω-6) and C18:3 (ω-3) were present in a ratio of 3:1. The Ensabi oil was primarily composed of long-chain-unsaturated fatty acids (LCUFA) accounting for 42% of the total fatty acids (**Table 6.5**).

According to FAO/WHO recommendation for dietary fats in human nutrition, the fatty acid composition of improved edible oil should have a high ratio of MUFA/ SFA and a significant proportion of two essential PUFAs, *i.e.* C18:2 (ω-6) and C18:3 (ω-3), with a desirable ratio between 5:1 and 10:1 (**Table 6.5**). Thus, Ensabi oil does not possess the ideal composition of fatty acids recommended for human nutrition. In particular, erucic acid is nutritionally undesirable (Ghafoorunissa 1998) and the high-eruate mustard oil is detrimental to mammalian health (Sinha *et al.* 2007).

The average protein content of Ensabi seeds was found as 32.1 % (**Table 6.2**) which makes it a potential source of food-grade vegetable protein.

The content of glucosinolates in Ensabi seeds was determined as 34.8 μmol g^{-1} (**Table 6.2**). In 1997, a new canola definition was scheduled to come into effect in Canada which included seed from the whole of the *Brassica* genus, if it contains less than 18 μmol of total glucosinolates g^{-1} whole seed at a moisture content of 8.5% (Uppstrom 1997). Ensabi oil with 34.8 μmol g^{-1} glucosinolate content can not be considered as a canola oil.

6.3.1.1 Electrophoretic characterization of *Brassica juncea* var. Ensabi

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of protein samples obtained at different growth stages of *B. juncea* var. Ensabi (seed, **S**; shoot of seedling, $\mathbf{SH_S}$; root of seedling, $\mathbf{R_S}$; shoot of the plant before flowering, $\mathbf{SH_{BF}}$; root of the plant before flowering, $\mathbf{R_{BF}}$; shoot of the plant after flowering, $\mathbf{SH_{AF}}$; root of the plant after flowering, $\mathbf{R_{AF}}$) showed presence of various proteins ranging from low to high molecular weights. **Table 6.6** shows molecular weight (MW) and corresponding relative mobility (R_m) values of different marker proteins which yielded a standard linear plot (**Figs. 6.4 b**, **6.5 b** and **6.6 b**) following the given straight line equation:

$$\text{Log MW} = -0.926\,(R_m) + 5.2 \qquad (2)$$

Molecular weights of various proteins at different growth stages of *B. juncea* var. Ensabi were computed from the above equation.

There were a total of 9 major protein bands in seed (**S**) sample ranging in molecular weight from 14,2953 to 22,706 Dalton (**Table 6.7**), in which proteins **S3**, **S4**, **S6**, **S7**, **S8** and **S9** were present at higher amounts (**Figs. 6.4**, **6.5** and **6.6**). The major proteins observed in seed sample may represent different naturally occurring enzymes, responsible for hydrolysis of food store in seed such as amylase, maltase, protease,

carbohydrase and lipase, biochemical reactions associated with germination and aerobic/anaerobic respiration. These proteins may also include the storage proteins.

When the seed (embryo) germinated as seedling, new proteins particularly of high molecular weights appeared both in shoot (**SH_S**) and root (**R_S**) of seedling (**Figs. 6.4 a** and **b**) due to gene up-regulation. These proteins include **SH_S1, 2, 3, 4, 5, 6, 8, 9, 10** and **11** and **R_S1, 2, 3, 4, 5, 6, 9** and **10** in shoot and root of seedling, respectively.

Table 6.6. Molecular weights and relative mobility values of different marker proteins.

Marker proteins	Molecular weight, MW (Dalton)*	Log MW	Relative mobility (R_m)
β-galactosidase	116,254	5.065	0.169
Bovine serum albumin (BSA)	84,796	4.928	0.290
Ovalbumin	53,896	4.732	0.468
Carbonic anhydrase	37,418	4.573	0.694
Soybean trypsin inhibitor	29,051	4.463	0.790
Lysozyme	19,809	4.297	0.984

*Calibrated molecular weights (Daltons) of the marker proteins were taken from technical bulletin of BIORAD Prestained SDS-PAGE Standards on Tris-HCl gel (Broad range) (Catalog No. 161-0318, Control 310004830).

New proteins emerged steadily for cell division, enlargement and differentiation to initiate growth of embryo root (radicle) and embryo shoot (plumule). However, emergence of high molecular weight proteins may also be the result of assembly of low molecular weight components. Molecular weights of proteins present in **SH_S** and **R_S** are tabulated in **Table 6.7**.

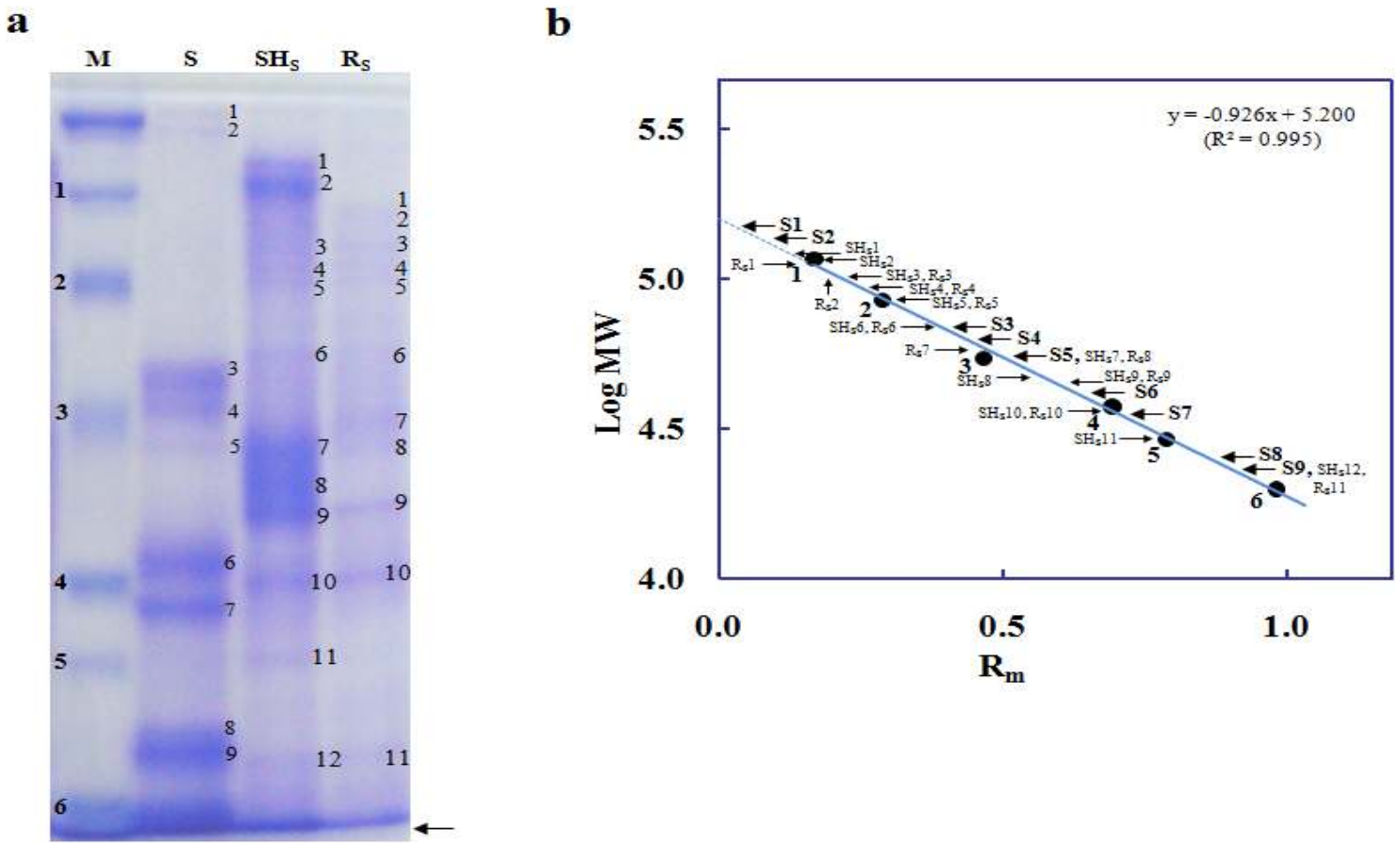

Fig. 6.4. (a) SDS-PAGE pattern of marker proteins **(M),** seed **(S),** shoot **(SH_S)** and root **(R_S)** samples of *Brassica juncea* var. Ensabi seedling stage performed according to the method of Laemmli (1970) on 10 % polyacrylamide gel. The arrow shows the position of the tracking dye, bromophenol blue. About 10 µl of sample containing 12 µg of protein was loaded in each well and electrophoresis was carried out in tris-glycine buffer, pH 8.3 containing 0.1 % SDS for 2 h. The gel was stained with 0.2 % (w/v) coomassie brilliant blue R-250 and destained in 5 % methanol, 7 % acetic acid solution. Marker proteins used were: 1. β-galactosidase; 2. BSA; 3. ovalbumin; 4. carbonic anhydrase; 5. soybean trypsin inhibitor and 6. lysozyme. Fractionated proteins in each preparation are numbered accordingly. **(b)** Determination of molecular weights of proteins. Numbers 1-6 refer to marker proteins as indicated in the legend to **Fig. 6.4a**. Positions of **S1-9**, **SH_S1-12** and **R_S1-11** are shown by arrows. Straight line was drawn using least squares analysis.

Table 6.7. Relative mobility (R_m) and molecular weight (MW) values of different protein bands obtained with seed and seedling (shoot and root) stage of *Brassica juncea* var. Ensabi.

Seed (S)			Shoot of seedling (SH_S)			Root of seedling (R_S)		
Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)
1	0.048	142,953	1	0.121	122,457	1	0.161	112,369
2	0.073	135,766	2	0.153	114,318	2	0.185	106,719
3	0.403	67,083	3	0.242	94,617	3	0.242	94,617
4	0.444	61,557	4	0.266	89,860	4	0.266	89,860
5	0.500	54,576	5	0.282	86,822	5	0.282	86,822
6	0.645	40,048	6	0.379	70,634	6	0.379	70,634
7	0.710	34,901	7	0.500	54,576	7	0.468	58,462
8	0.871	24,745	8	0.556	48,387	8	0.500	54,576
9	0.911	22,706	9	0.597	44,400	9	0.597	44,400
			10	0.685	36,749	10	0.685	36,749
			11	0.782	29,897	11	0.911	22,706
			12	0.911	22,706			

= Protein bands similar to those present in seed.

Blue colored digits indicate similar proteins in shoot and root samples of *Brassica juncea* var. Ensabi seedling

Proteins **S5** remained expressed in shoot and root sample of seedling as **SH_S7** and **R_S8**, respectively. Similarly, **S9** was also present as **SH_S12** and **R_S11**. Nevertheless, genes responsible for expression of **S1**, **S2**, **S3**, **S4**, **S6**, **S7** and **S8** proteins in seed seem to be down-regulated once the seed transforms into seedling. This is possibly due to proteins (enzymes) needed for catabolic reaction in storage centre are no longer required as the dry mass of food storage declines during germination (Murthy and Sun 2000). Proteins **SH_S3**, **4**, **5**, **6**, **7**, **9**, **10** and **12** were found to be similar to **R_S3**, **4**, **5**, **6**, **8**, **9**, **10** and **11**, respectively. **SH_S1**, **2**, **8** and **11** and **R_S1**, **2** and **7** were the distinctive proteins present in shoot and root of seedling, respectively. **SH_S1**, **2**, **8** and **11** may represent the soluble enzymes available in leaves (plumule) particularly in chloroplast and stroma which are responsible for photosynthesis process in the leaves.

Figure 6.5 a shows SDS-PAGE pattern of proteins present in shoot (**SH_{BF}**) and root (**R_{BF}**) of *Brassica juncea* var. Ensabi before flowering stage. Protein **S9** (**SH_S12** and **R_S11** in seedling) still remained present in shoot and root before flowering stage as **SH_{BF}8** and **R_{BF}9**, respectively (**Table 6.7**). Protein **S5 (SH_S7** and **R_S8** in seedling) was also present as **SH_{BF}5** and closer to **R_{BF}5** (**Figs. 6.5 a** and **b**, **Table 6.8**). Proteins in seedling such as **SH_S1**, **SH_S11**, **R_S2** and **R_S10**, remained akin in *B. juncea* var. Ensabi before flowering stage as **SH_{BF}2**, **SH_{BF}7**, **R_{BF}1** and **R_{BF}7**, respectively (**Tables 6.7** and **6.8**). These are the proteins from seedling which possibly support continuous elongation of cells, growth of leaves and roots (lateral) of the plant during the primary growth stage of plant body as well as enzymes responsible for photosynthesis. However, bands **SH_S2**, **3**, **4**, **5**, **6**, **8**, **9**, **10** and **R_S1**, **3**, **4**, **5**, **6**, **7**, **9** disappeared when the seedling transformed into plant before flowering stage.

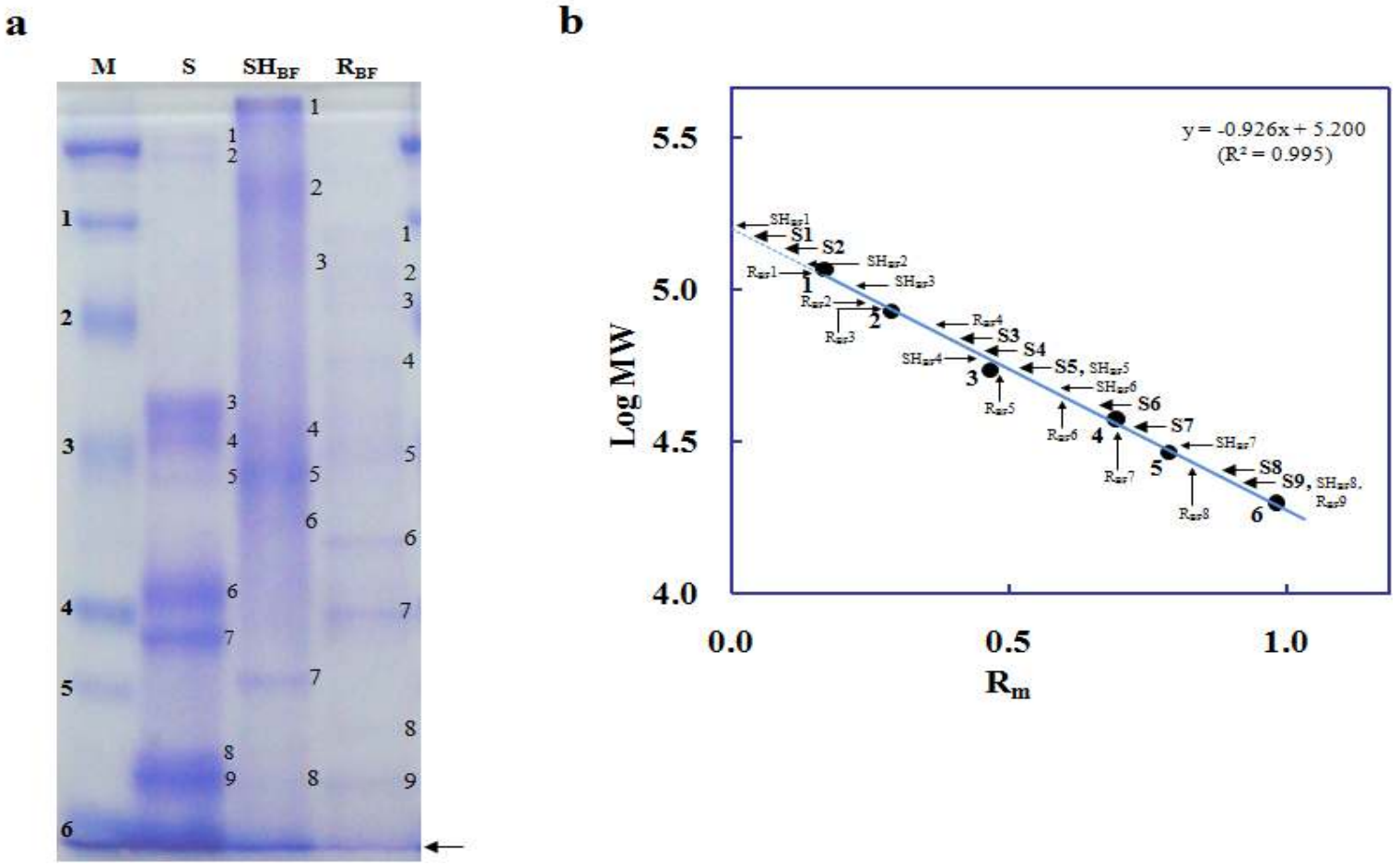

Fig. 6.5. (a) SDS-PAGE pattern of marker proteins **(M),** seed **(S),** shoot **(SH_{BF})** and root **(R_{BF})** samples of *Brassica juncea* var. Ensabi before flowerin stage performed according to the method of Laemmli (1970) on 10 % polyacrylamide gel. The arrow shows the position of the tracking dye, bromophenc blue. About 10 µl of sample containing 12 µg of protein was loaded in each well and electrophoresis was carried out in tris-glycine buffer, pH 8. containing 0.1 % SDS for 2 h. The gel was stained with 0.2 % (w/v) coomassie brilliant blue R-250 and destained in 5 % methanol, 7 % acetic aci solution. Marker proteins used were: 1. β-galactosidase; 2. BSA; 3. ovalbumin; 4. carbonic anhydrase; 5. soybean trypsin inhibitor and 6. lysozyme Fractionated proteins in each preparation are numbered accordingly. **(b)** Determination of molecular weights of proteins. Numbers 1-6 refer to marke proteins as indicated in the legend to **Fig. 6.5 a**. Positions of **S1-9**, **SH_{BF}1-8** and **R_{BF}1-9** are shown by arrows. Straight line was drawn using least square analysis.

Table 6.8. Relative mobility (R_m) and molecular weight (MW) values of different protein bands obtained with seed, shoot and root before-flowering stage of *Brassica juncea* var. Ensabi.

Seed (S)			Shoot before-flowering (SH_{BF})			Root before-flowering (R_{BF})		
Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)
1	0.048	142,953	1	0.015	153,375	1	0.185	106,917
2	0.073	135,766	2	0.123	121,908	2	0.231	96,897
3	0.403	67,083	3	0.215	100,128	3	0.292	84,981
4	0.444	61,557	4	0.454	60,220	4	0.362	73,319
5	0.500	54,576	5	0.500	54,576	5	0.485	56,396
6	0.645	40,048	6	0.569	47,086	6	0.600	44,096
7	0.710	34,901	7	0.785	29,747	7	0.700	35,629
8	0.871	24,745	8	0.911	22,706	8	0.838	26,521
9	0.911	22,706				9	0.911	22,706

= Protein bands similar to those present in seed.

Red colored digits indicate similar proteins in shoot and root of *Brassica juncea* var. Ensabi before flowering stage.

Proteins **$SH_{BF}1$**, **$SH_{BF}3$**, **$SH_{BF}4$**, **$SH_{BF}6$**, **$R_{BF}2$**, **$R_{BF}3$**, **$R_{BF}4$**, **$R_{BF}6$** and **$R_{BF}8$** were the new proteins present in shoot and root, respectively before flowering stage compared to seedling. New proteins in shoot may include proteins responsible for initial growth of lateral buds and reproductive organs (flowers) of the plant. All the proteins found in **SH_{BF}** were not comparable to proteins present in **R_{BF}** (except **$SH_{BF}8$** and **$R_{BF}9$**) (**Table 6.8**) as compared to the condition in seedling (**Table 6.7**).

Protein **S5** and **S9** were still retained in shoot and root of *B. juncea* var. Ensabi even after the flowering stage (**Figs. 6.6 a** and **b**, **Table 6.9**) as **$SH_{AF}5$** and **$R_{AF}5$** as well as **$SH_{AF}9$** and **$R_{AF}8$**, respectively. These proteins may possibly represent the enzymes responsible for respiratory reactions occurring in the plant from the very early seed stage. New proteins which appeared in the seedling such as **SH_S11**, **R_S2** and **R_S10** (**$SH_{BF}7$**, **$R_{BF}1$** and **$R_{BF}7$**) were still remained in the after-flowering stage as **$SH_{AF}8$**, **$R_{AF}1$** and **$R_{AF}7$**, respectively (**Tables 6.7, 6.8** and **6.9**). Unique proteins which appeared in before-flowering stage such as **$R_{BF}2$** and **$R_{BF}6$** were remained in the after-flowering stage too as **$R_{AF}2$** and **$R_{AF}6$**, respectively (**Tables 6.8** and **6.9**). These proteins could be the components responsible for generation of flower. However, **$SH_{BF}1$**, **3**, **4** and **$R_{BF}2$**, **3**, **4**, **6**, **8** disappeared in the after-flowering stage. Proteins **$SH_{AF}1$**, **$SH_{AF}3$**, **$SH_{AF}4$**, **$SH_{AF}7$**, **$R_{AF}2$**, **$R_{AF}3$**, **$R_{AF}4$** and **$R_{AF}6$** were the new proteins present in shoot and root, respectively in the after-flowering stage compared to seedling and before-flowering stages (**Tables 6.7**, **6.8** and **6.9**). Proteins **$SH_{AF}2$**, **3** and **4** were found to be similar to **$R_{AF}1$**, **2** and **3**, respectively in this stage (**Table 6.9**).

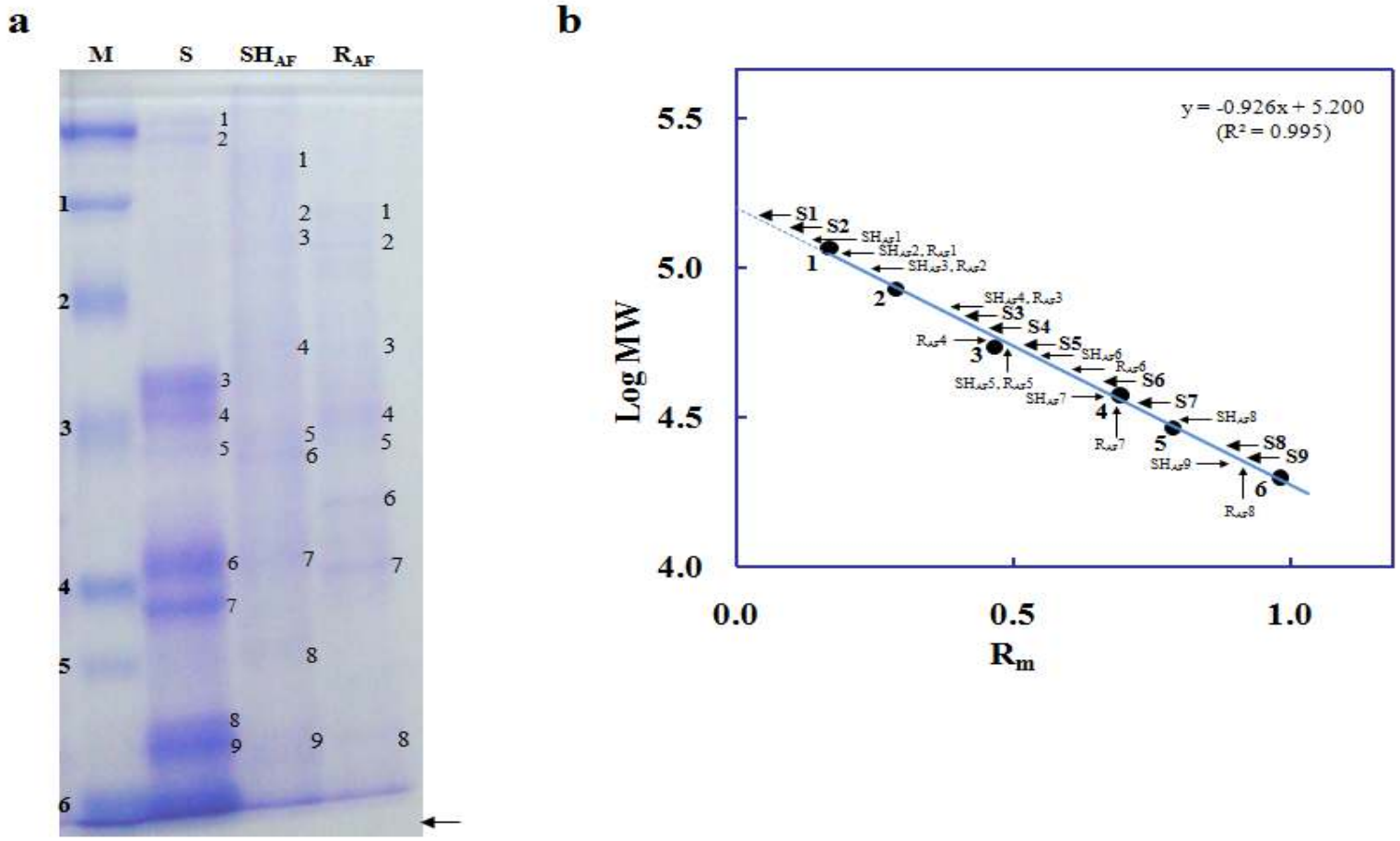

Fig. 6.6. (a) SDS-PAGE pattern of marker proteins **(M),** seed **(S),** shoot **(SH_{AF})** and root **(R_{AF})** samples of *Brassica juncea* var. Ensabi after flowering stage performed according to the method of Laemmli (1970) on 10 % polyacrylamide gel. The arrow shows the position of the tracking dye, bromophenol blue. About 10 µl of sample containing 12 µg of protein was loaded in each well and electrophoresis was carried out in tris-glycine buffer, pH 8.3 containing 0.1 % SDS for 2 h. The gel was stained with 0.2 % (w/v) coomassie brilliant blue R-250 and destained in 5 % methanol, 7 % acetic acid solution. Marker proteins used were: 1. β-galactosidase; 2. BSA; 3. ovalbumin; 4. carbonic anhydrase; 5. soybean trypsin inhibitor and 6. lysozyme. Fractionated proteins appeared in each preparation are numbered accordingly. **(b)** Determination of molecular weights of proteins. Numbers 1-6 refer to marker proteins as indicated in the legend to **Fig. 6.6 a**. Positions of **S1-9**, **SH_{AF}1-9** and **R_{AF}1-8** are shown by arrows. Straight line was drawn using least squares analysis.

Table 6.9. Relative mobility (R_m) and molecular weight (MW) values of different protein bands obtained with seed, shoot and root after-flowering stage of *Brassica juncea* var. Ensabi.

Seed (S)			Shoot after-flowering (SH_{AF})			Root after-flowering (R_{AF})		
Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)	Protein band	R_m	MW (Da)
1	0.048	142,953	1	0.109	125,522	1	0.188	106,261
2	0.073	135,766	2	0.188	106,261	2	0.234	96,154
3	0.403	67,083	3	0.234	96,154	3	0.383	70,067
4	0.444	61,557	4	0.383	70,067	4	0.469	58,336
5	0.500	54,576	5	0.492	55,493	5	0.492	55,493
6	0.645	40,048	6	0.531	51,058	6	0.594	44,688
7	0.710	34,901	7	0.672	37,831	7	0.688	36,591
8	0.871	24,745	8	0.781	29,962	8	0.922	22,200
9	0.911	22,706	9	0.906	22,952			

= Protein bands similar to those present in seed.

Purple colored digits indicate similar proteins in shoot and root of *Brassica juncea* after flowering stage.

6.3.2 Allaelopathic activity of *B. juncea* var. Ensabi

6.3.2.1 Aqueous extracts

Effect of different concentrations of aqueous extracts of dried and fresh plant parts (root, stem and leaf) of *B. juncea* var. Ensabi on rate of germination, mean period of final germination, final germination (%), radicle and shoot lengths of two target plant species (*E. crus-galli*, Beauv. var. oryzicola Ohwi and *R. sativus* L.) are shown in **Tables 6.10** and **6.11**. Results showed significant differences (p <0.05) between different plant parts on seed germination of radish and barnyard grass when treated with 200 gL^{-1} of *B. juncea* var. Ensabi aqueous extracts isolated from dried root, stem and leaf parts (**Fig. 6.7**).

6.3.2.1.1 Effect of different aqueous extracts on radish seeds' germination

Analysis of variance and Tukey' test (HSD) for radish seeds showed that the application of 300 gL^{-1} aqueous extract of dried plant parts had completely abolished the germination. Both aqueous extracts obtained from fresh (≥ 200 gL^{-1}) and dried (≥ 120 gL^{-1}) plant parts produced significant (p<0.05) inhibitory effect in a concentration dependent manner. However, the effect was more pronounced with aqueous extract of dried plant parts compared to that obtained with fresh plant parts (**Table 6.10**). Application of 50 and 40 gL^{-1} of aqueous extract of fresh and dried plant parts respectively had no effect on radish seeds germination, as the value was similar to that obtained with control (**Table 6.10**).

Aqueous extract of dried plant parts in concentrations of 40 and 80 gL^{-1} of *B. juncea* var. Ensabi significantly increased the root length of radish early seedlings. This effect was similar to the one obtained with the application of 150 gL^{-1} fresh plant parts' aqueous extract. Increase in concentration of aqueous extract of dried plant parts in the range, 120-300 gL^{-1} significantly reduced the root length. Complete inhibition of both root and shoot lengths was observed with 300 gL^{-1}

concentration of dried plants' extract (**Table 6.10**). The inhibition effect was less pronounced in aqueous extract of fresh plant parts.

6.3.2.1.2 Effect of different aqueous extracts on barnyard grass seeds' germination

The experimental data on seed germination of barnyard grass (*E. crus-galli*, Beauv. var. *oryzicola* Ohwi) showed 87% germination with the use of distilled water as a control. Application of increasing concentrations of aqueous extracts from both fresh and dried plant parts (root, stem and leaf) significantly inhibited the germination of barnyard grass seeds. The inhibition was smaller at lower concentrations 50-150 gL^{-1} for fresh plants and 40-80 gL^{-1} for dried plants extracts but became more pronounced at higher concentrations (**Table 6.11**).

Aqueous extract from dried plant parts was more effective in inhibiting the seed germination compared to the extract from fresh plant parts as ~ 94% inhibition was observed with the use of 300 gL^{-1} of aqueous extract of dried plant parts whereas only 46% inhibition was obtained with fresh plants aqueous extract with the same concentration. These results were similar to those reported earlier (Ahn and Chung 2000; Chung *et al.* 2003; Chaves *et al.* 2001; Asghari and Tewari 2007).

Root length of early seedlings of barnyard grass increased significantly (28%) with the application of 50 gL^{-1} of aqueous extract of fresh plant parts of *B. juncea* var. Ensabi. Use of higher concentrations of aquoeus extracts from both fresh and dried plants parts showed inhibition in root growth which increased with the increase in aquoeus extract concentration being more pronounced with dried plants aqueous extract compared to fresh plants' aqueous extract (**Table 6.11**).

Table 6.10. Effect of *Brassica juncea* var. Ensabi aqueous extracts of whole plant parts (root, stem and leaf) on germination parameters of radish.

	Aqueous extract concentration ($g.L^{-1}$)	Rate of germination (seeds day^{-1})	Mean period of final germination (days)	Final germination (%)	Germination inhibition / stimulation (%)	Root length (cm)	Shoot length (cm)
Control	0.	24.7 a*	1.5 d	94.4 ab	0.0	8.7 ab	4.2 bcd
Aqueous extract (fresh plants) (gL^{-1})	50.	24.4 a	1.5 d	95.2 a	-0.8	8.7 ab	4.4 abcd
	100	22.6 bc	1.5 cd	93.3 ab	1.2	8.4 abc	3.8 cde
	150	22.1 c	1.5 cd	91.5 ab	3.1	9.3 a	4.9 abc
	200	19.7 d	1.5 d	81.5 c	13.7	6.5 bcd	5.5 ab
	300	13.2 f	1.7 c	58.5 e	38.0	6.0 cd	5.8 a
Aqueous extract (dried plants) ($g.L^{-1}$)	40	23.8 ab	1. 4 d	96.7 a	-1.7	9.4 a	4.9 abc
	80	21.4 c	1.6 cd	89.3 b	5.5	10.0 a	5.5 ab
	120	15.6 e	1.8 c	68.5 d	27.5	6.0 cd	4.3 bcd
	160	8.7 g	2.4 b	48.2 f	49.0	5.1 d	3.3 de
	200	3.9 h	3.3 a	22.2 g	76.5	7.6 abcd	2.7 e
	300	0.7 i	2.5 b	0.0 h	100.0	0.0 e	0.0 f
Sx , at alpha = 0.05		0.3	0.04	1.2		0.5	3.1

* Means followed by the same letter are not significantly different at $p < 0.05$.

A comparison on the effect of aqueous extracts on root and shoot growth suggests that the root length of barnyard grass was relatively more sensitive to different concentrations of aqueous extracts from both fresh and dried plant parts compared to shoot length. Such differences can be ascribed to the early absorption of allelochemicals or autotoxic compounds by the roots from the environment (Turk and Tawaha 2002). These results were in agreement with earlier reports suggesting that water extracts of allelopathic plants had more special effect on root growth than on shoot growth (Chung and Miller 1995; Turk and Tawaha 2003, Turk *et al.* 2005). The seed germination of radish and barnyard grass seeds were investigated using soluble extracts from different parts (root, stem and leaves) of *B. juncea* var. Ensabi (**Fig. 6.7**). Results showed the effect of aqueous extracts of different plant parts on the target weeds' seed germination. Significant differences were noted in both radish and barnyard grass seed germination upon application of these extracts. While both stem and leaf extracts showed similar effect on seed germination, root extract was more effective against barnyard grass compared to radish seed germination.

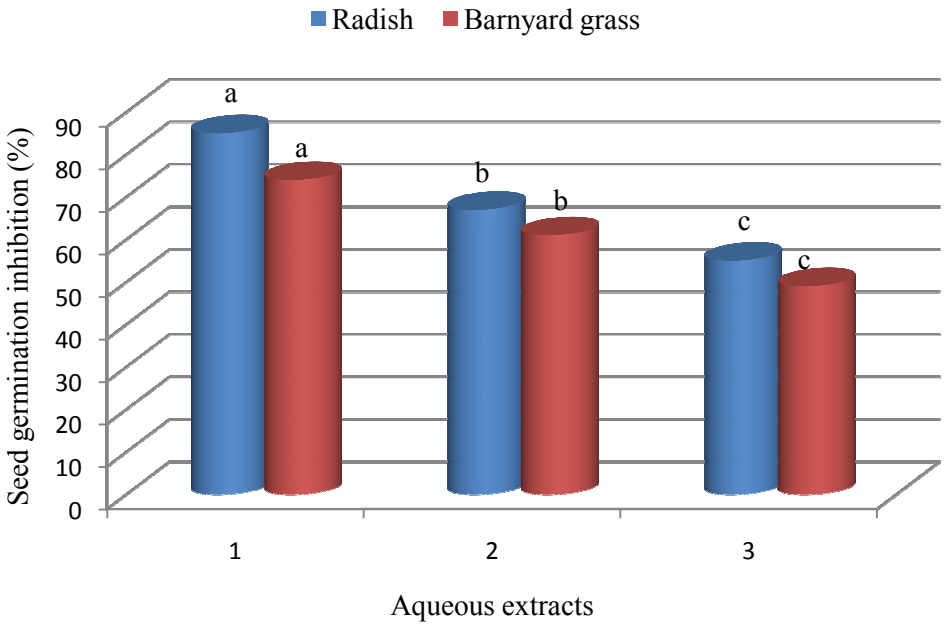

Fig. 6.7. Effect of aqueous extracts (200 gL^{-1}) from different dried plant parts on inhibition of seed germination (%) of radish and barnyard grass: 1- Leaf extract, 2- Stem extract, 3- Root extract.

Fig. 6.8. Consumptive root and shoot lengths of barnyard grass when treated with different concentrations of *Brassica juncea* var. Ensabi extracts, isolated using ethanol solvent. The seedling (a) was treated with distilled water (control).

Table 6.11. Effect of *Brassica juncea* var. Ensabi aqueous extracts of whole plant parts (root, stem and leaf) on germination parameters of barnyard grass.

	Aqueous extract concentration ($g.L^{-1}$)	Rate of germination (seeds day^{-1})	Mean period of final germination (days)	Final germination (%)	Germination inhibition (%)	Root length (cm)	Shoot length (cm)
Control	0	7.4 a	3.7 b	87.0 a	0.0	6.8 b	2.4 b
Aqoueous extract (fresh plants) (gL^{-1})	50	6.3 c	4.1 ab	80.0 abc	8.1	8.7 a	2.9 ab
	100	6.9 b	3.9 b	83.3 ab	4.3	7.2 ab	2.7 ab
	150	5.9 c	4.1 ab	77.0 bc	11.5	7.4 ab	2.6 ab
	200	4.1 e	4.9 ab	65.9 d	24.3	4.7 cd	3.7 a
	300	2.7 f	5.5 a	47.4 f	45.5	3.2 def	3.3 ab
Aqoueous extract (dried plants) (gL^{-1})	40	6.2 c	4.2 ab	80.4 abc	7.7	5.0 c	2.6 ab
	80	6.0 c	4.17 ab	75.2 c	13.6	3.7 cde	2.5 b
	120	5.0 d	4.3 ab	67.8 d	22.1	2.9 ef	2.6 ab
	160	4.1 d	4.4 ab	57.0 e	34.5	1.8 fg	2.9 ab
	200	2.9 f	5.0 ab	44.8 f	48.5	1.2 gh	3.3 ab
	300	0.3 g	3.7 b	5.6 g	93.6	0.0 g	0.0 c
Sx , at alpha = 0.05		0.1	0.3	1.5		0.7	0.3

6.3.2.2 Ethanol extract

Results showed that the allelopathic activity of ethanol extracts was significantly (p <0.05) similar to that of aqueous extracts in different plant parts on seed germination and seedlings of radish and barnyard grass.

6.3.2.2.1 Effect of different ethanol extracts on radish seeds' germination

Results showed all ethanol extracts obtained from the leaves, stems and roots of *Brassica juncea* var. Ensabi significantly (p<0.05) inhibited the germination of radish seed. The germination inhibition was highest (100%) at ethanol extract concentration of 30 gL^{-1}. Increase in concentration of the extracts led to a significant (p<0.05) decrease in final germination from 91% in the control to the lowest value (0.0%) with the application of highest ethanol extract concentration (**Table 6.12**).

Results revealed increasing concentrations of root, stem and leaf extracts significantly decreased the rate of germination from 24.3 to 0.0 seeds day^{-1} for control and with the application of 30 gL^{-1} of ethanol extract respectively (**Table 6.12**). The highest value of mean period of final germination was observed at the concentration of ethanol extract of 18.0 gL^{-1}.

Ethanol extract concentration of 18.0 gL^{-1} inhibited the root length (83.7%) and shoot length (79%) of radish compared with the control. Chemical extracts impeded the germination and caused abnormal root elongation and seedling abnormality. Analysis of variance indicated that ethanol extract did not influence significantly p<0.05 the total seedling dry matter.

Table 6.12. Effect of *Brassica juncea* var. Ensabi ethanol extracts of whole plant parts (root, stem and leaf) on germination parameters of radish.

	Ethanol extract concentration (gL^{-1})	Rate of germination (seeds day^{-1})	Mean period of final germination (days)	Final germination (%)	Germination inhibition (%)	Root length (cm)	Shoot length (cm)
Control	0.0	24.3 a*	2.3 cd	91.0 a	---	9.8 a	4.3 a
Ethanol extract	10.8	10.4 b	3.5 c	77.6 a	14.7 c	4.1 b	3.0 b
	14.3	6.7 c	3.8 b	57.3 b	37.0 b	2.3 c	2.7 b
	18.0	2.3 d	4.7 a	23.0 c	74.7 a	1.6 d	0.9 c
	30.0	0.0 e	0.0	0.0 d	100.0 a	0.0 d	0.0 c
Sx , $p< 0.05$		0.9	0.3	7.9	---	0.4	0.4

* Means followed by the same letter are not significantly different at $p < 0.05$.

6.3.2.2.2 Effect of different ethanol extracts on barnyard grass seeds' germination

Results of this experiment indicated that germination of barnyard grass (*E. crus-galli*, Beauv. var. oryzicola Ohwi) seed was inhibited significantly ($p < 0.05$) when treated with ethanol extract of the leaves or stem of *B. juncea* var. Ensabi (**Table 6.13**). Final germination percentage of barnyard grass decreased significantly ($p < 0.05$) from 87.5 % with the application of distilled water (control) to 0.0 % with application of 30 gL^{-1} ethanol extract of plant parts (**Table 6.13**).

Results indicated that the rate of germination (RG) was 7.1 seeds day^{-1} with the control. Increasing concentrations of root and shoot ethanol extract significantly decreased RG. The application of 10.8 gL^{-1} ethanol extract reduced the RG to 4.7 seeds day^{-1} while ethanol extract concentrations of 14.3 gL^{-1} had no significant effect ($p < 0.05$) on the RG. **Table 6.13** shows that the mean period of final germination increased in response to application of ethanol extract. However, mean period of final germination differences were not significant at $p < 0.05$. The same results were reported on allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination (Jefferson and Pennacchio 2003). Mean period of final germination with control was 4.1 days, which increased to 7.3 days with the application of 18 gL^{-1} plant parts' extract.

Results revealed, increasing concentrations of *B. juncea* var. Ensabi plant parts' ethanol extract significantly reduced root and shoot lengths of barnyard grass (**Table 6.13**). The root systems, especially root tips of barnyard grass were stunted, swollen and shortened (92.0%) by ethanol extract at 18 gL^{-1} concentration.

Table 6.13. Effect of ethanol extracts of *Brassica juncea* var. Ensabi on seed germination in barnyard grass (RG = Rate of germination, MPFG= Mean period of final germination, FG = Final germination).

	Ethanol extract concentration ($g.L^{-1}$)	Rate of germination (seeds day^{-1})	Mean period of final germination (days)	Final germination (%)	Germination inhibition (%)	Root length (cm)	Shoot length (cm)
Control	0.0	7.1 a*	4.1	87.5 a	0.0	5.1 a	4.3 a
Ethanol extract	10.8	4.7 b	5.1	65.8 b	24.8	3.2 b	3.7 a
	14.3	3.8 b	6.1	49.3 c	43.7	1.5 c	2.6 bc
	18.0	2.72 b	7.3	38.9 d	55.6	0.4 cd	0.6 c
	30.0	0.0 c	0.0	0.0 e	100.0	0.0 cd	0.0 d
Sx, $p<0.05$		0.4	0.3	4.3		0.3	0.1

* Means followed by the same letter are not significantly different at $p<0.05$.

Both root and shoot elongation of germinated seedlings was inhibited by ethanol extracts. The ethanol extract of *B. juncea* var. Ensabi was even more potent and inhibited radish seeds.

Allelopathy can affect many aspects of plant ecology including occurrence, growth, plant succession, the structure of plant communities, dominance, diversity and plant productivity. Plants that germinate at slower rates are often smaller. This may seriously influence their chances of competing with neighbouring plants for resources such as water, especially in arid or semi-arid regions (Faravani 2008).

The allelochemicals present in *B. juncea* var. Ensabi extracts might have appeared to inhibit or stunt the growth of roots and shoots of germinates by at least two mechanisms. The reason is the existence of phenolic compounds. These were identified and separated from *B. juncea* var. Ensabi extracts (Dafaalla 2004) and possibly, they inhibited root elongation and cell division completely, indicating that the thickness of seminal roots was enlarged abnormally. It is thought that only transverse growth of root was sequentially maintained while longitudinal growth was greatly inhibited by the extracts and the phenolic (Chon *et al.* 2002). These phenomena may account for the results obtained in the study with the extracts of *B. juncea* var. Ensabi.

This research suggests that *B. juncea* var. Ensabi plant extracts significantly affected root growth and morphological differentiation of susceptible plants. Thus, the result on growing plants will be a reduction of plant biomass in the presence of either autotoxic or allelopathic compounds. These results may have value in enabling weed control based on natural plant extracts. We have demonstrated that allelochemicals are produced in the shoot, root and leaf of *B. juncea* var. Ensabi. Such chemicals are both species-specific and concentration-dependent and these characteristics may influence the density and the composition of individual plant communities. Allelochemicals may directly prevent or promote germination when environmental conditions are conducive to growth

and establishment, therefore, influencing the number of plants of each species in a community (Jefferson and Pennacchio 2003).

6.3.2.2 Sandwich method

Application of the 'sandwich method' for the evaluation of allelopathic activity of leaf, stem and root materials of *B. juncea* var. Ensabi is shown in **Table 6.14**. Based on Fujii *et al*. (2004) for the evaluation of allelopathic activity, we introduced the concept of 'standard deviation value'. For statistical analysis, we calculated the mean (M) and standard deviation (ó), and evaluated the criteria of the standard deviation value (SDV). Among the different parts tested, leaf in amount of 50 mg showed strongest inhibitory activity compared to other plant parts and caused 100% growth inhibition in the radicle and hypocotyl in lettuce seedlings (**Figs. 6.9** and **6.10**). Stem in amount of 50 mg also showed strong inhibitory activity. Leaf in amount of 10 mg showed moderate inhibitory activity. Compared to these parts, stem in 10 mg and root in 50 and 10 mg, showed either no inhibitory or slight promotive activity. Sandwich method is very useful and requires only a short period to elucidate the allelopathic effect of leaf litter leachates when screening a large number of samples under laboratory conditions.

Table 6.14. Evaluation of allelopathic activity of dried leaf, stem and root materials of *Brassica juncea* var. Ensabi by the sandwich method.

Plant parts	Weight of plant parts (mg)	Radicle	Hypocotyl
Leaf	50	0**	0**
Leaf	10	37	64
Stem	50	22*	47*
Stem	10	59	83
Root	50	55	86
Root	10	90	100

Radicle and hypocotyl refer to the percentage growth of lettuce seedlings compared to the control (in agar medium). *, Inhibitory activity stronger than the standard deviation of low value; **, inhibitory activity stronger than the standard deviation of high value.

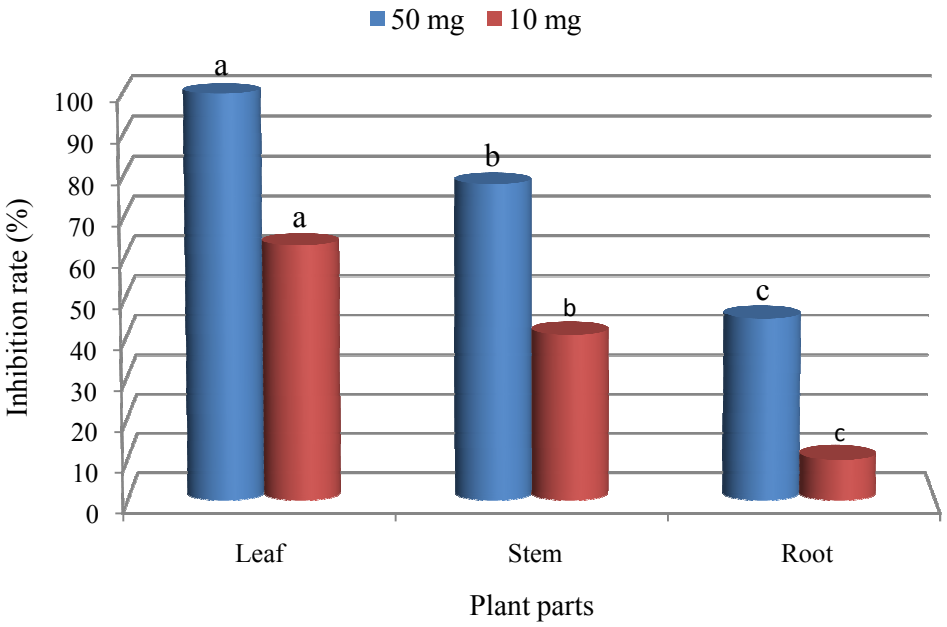

Fig. 6.9. Effect of dried leaf, stem and root materials of *Brassica juncea* var. Ensabi on the growth inhibition of radicle of lettuce seedlings by sandwich method.

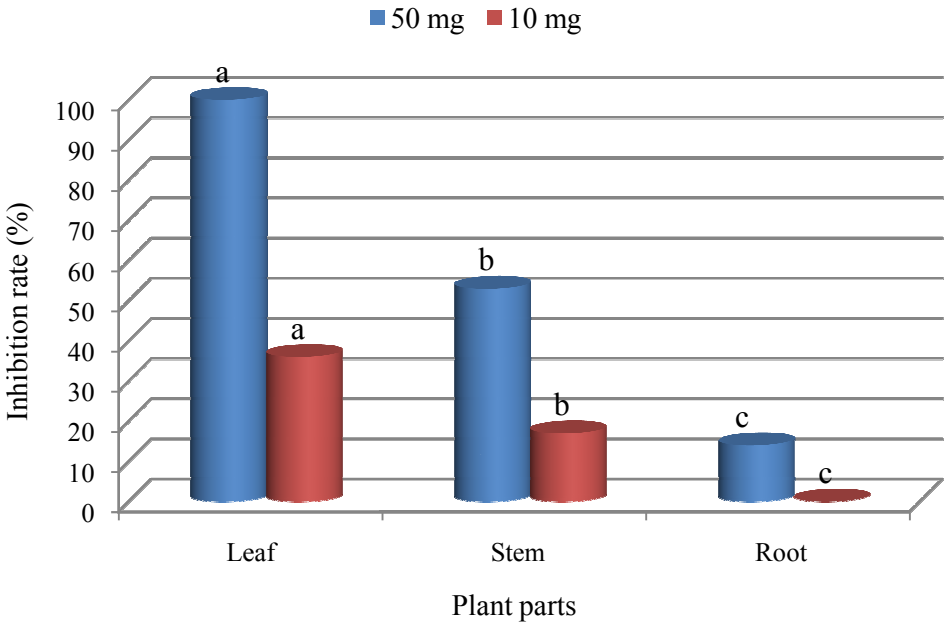

Fig. 6.10. Effect of dried leaf, stem and root materials of *Brassica juncea* var. Ensabi on the growth inhibition/stimulation of hypocotyl of lettuce seedlings by sandwich method.

CHAPTER 7

GENERAL DISCUSSION AND CONCLUSION

The wild Malaysian brassica (*B. juncea* var. Ensabi) with its distinct pungent aroma and bitter taste is now cultivated as a local vegetable among the Malays and natives of Sabah and Sarawak. The species is also used as a popular kimchi by the locals.

The present series of study encompasses:

1. To study the environmental effects (*e.g.* temperature, light, drought stress, salinity, etc) on seed germination and seedling growth of Ensabi.
2. To determine effect of N, P, K on some morphological characters, yield and yield components of Ensabi.
3. To identify of ecological affects of plant population on general growth pattern and intraspecific-competition to understand the mechanisms of competition at the individual plant level of Ensabi.
4. To study agronomic traits and allelopathic effect of Ensabi.
5. To assessment the chemical nature of Ensabi.

The results of this study are the first comprehensive report on *B. juncea* var. Ensabi, and as such contribute to the knowledge on some selected topics in plant population and production ecology as the initial steps to help understand the essential processes and interactions that lead to small- and large-scale hierarchical structures in plant population or communities. Further, the understanding of such processes and ensuing interactions may also help to explain allometry and growth patterns in plants either of the same cohort or otherwise, especially under the influence of neighbours, herbivory and pathogens. A brief summary of the findings is reported here. Major findings have been discussed earlier in each chapter in details.

Seed germination is an important stage in the life history of plant, affecting seedling development and survival, and population dynamics of any plant species. Seed germination can be defined as the growth of the embryo of the mature seed into a stage capable of continuing

growth into a mature plant. Such growth is very much dependent on the immediate environmental conditions such as water and oxygen availability as well as temperature regime(s) to which the seeds are exposed

Brassica juncea var. Ensabi did not show dormancy, as the seeds germinated immediately when placed under appropriate or favourable germination conditions. Germination capacity declined at 35 ºC, revealing 25 ºC as the optimum germination temperature for visible signs of germination, and this occurred from 1 to 4 days after sowing, being earlier at higher temperatures of 30-35 ºC, but the highest and most complete germination occurred at 25-30 ºC (Chapter 2). The mean time to germination (MTG) and the rate of germination (RG) changed under different temperatures. The MTG was highest at 25-30^{o}C and declined at temperatures lower than 20^{o}C or above 35^{o}C. Temperature may affect either the initial processes of water uptake by seeds, or the ensuing biochemical processes, which result in cell division.

Germination and seedling establishment are critical stages in the life cycle of a plant especially under adverse environmental conditions (Ashraf *et al.* 2004). Salt stress reduced seed germination and also delayed the emergence of seeds in *B. juncea* var. Ensabi. It is also assumed that in addition to toxic effects of certain ions, higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds, and thus reduces germination (Jamil *et al.* 2006). It appears that a decrease in germination is related to salinity-induced disturbance of metabolic process leading to increase in phenolic compounds.

It is assumed that germination rate and the final seed germination decrease with the decrease of the water movement into the seeds during imbibitions (Jamil and Rha 2004). Salinity stress can affect seed germination through osmotic effects. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity. Germination percentage was also decreased significantly as the level of salinity of the medium increased. The mean time to

germination of *B. juncea* var. Ensabi increased with the addition of NaCl and this increasing was greater in higher concentration as compared to low concentration.

The root and shoot lengths are the most important parameters affected by salt stress because roots are in direct contact with soil and absorb water from soil and supply it to the rest of the plant. For this reason, root and shoot length provides an important clue to the response of plants to salt stress (Jamil and Rha 2004). Salt stress inhibited the seedling growth (root and shoot length of seedlings) but root length was more affected then shoots length (Chapter 2). Inhibition of plant growth by salinity may be due to the inhibitory effect of ions. The reduction in root and shoot development may be due to toxic effects of the NaCl used as well as unbalanced nutrient uptake by the seedlings. It may be due to the ability of the root system to control entry of ions to the shoot is of crucial importance to plant survival in the presence of NaCl (Hajibagheri *et al.* 1989). High salinity may inhibit root and shoot elongation due to slowing down of water uptake by the plant may be another reason for this decrease. Salinity can rapidly inhibit root growth and hence capacity of water uptake and essential mineral nutrition from soil. Salt stress inhibited the growth of root more than in *B. juncea* var. Ensabi. They observed that the root growth of safflower was more adversely affected compared to shoot growth. Hussain and Rehman (1997) reported that the roots of seedlings were more sensitive than the shoots. Some researchers argue that the plants had the reduction in their fresh weights because of the proportional increase in Na^+ concentration, which could imply that an ionic effect was being manifested. However, one could argue that because dry weights were not much affected compared to the fresh weights, growth reduction would be attributable to osmotic effects.

It has been reported that salinity has negative relationship with germination, root length, seedling shoot length, fresh seedling root and shoot weight. Zeynalabedin and Jafari (2002) reported that there was a negative relationship between salinity and germination, length of radicle and

plumule and also positive and significant correlation between percent of germination and length of radicle and plumule.

Drought is a major abiotic stress that plants encounter and can be responsible for the inhibition or delayed seed germination, poor seedling growth, and establishment. The NaCl, PEG (Poly Ethylene Glycol) also adversely affected the germination and seedling growth of *B. juncea* var. Ensabi but PEG had a greater inhibitory effect than NaCl. Murillo-Amador *et al*. (2002) observed that NaCl had a lower effect on the germination and seedling growth of cowpea than did PEG, and Sadeghian and Yavari (2004), who stated that seedling growth was severely diminished by water stress in sugar beet. Mean germination time varied with solutions and doses.

The first physiological disorder, which takes place during germination, is the reduction in imbibitions of water by seeds which leads to a series of metabolic changes, including changed enzyme activities and general reduction in hydrolysis and utilization of the seed reserve (Ahmad and Bano 1992). Upon imbibition, the quiescent dry seeds rapidly resume oxygen uptake and oxidative phosphorilation, processes required for supporting the high energy cost of germination (Baranova *et al.* 2006).

Osmotic stress examined in this work reduced the germination percentage of *B. juncea* var. Ensabi in respect to the concentration of PEG used. PEG, which is a non-penetrating agent, affects seed germination only by compromising water uptake. The marked differences in germination percentages observed with NaCl and PEG at the same osmotic potentials indicate specific ionic effects and point that germination is solely controlled by the osmotic potential.

The present study clearly demonstrates that the germination of *B. juncea* var. Ensabi seeds was influenced by the concentrations and even more by the nature and interactions of the ions present in the solutions. This suggests that the germination response is different when many salts are present, indicating that the toxic effect of NaCl can be considerably alleviated in nature by the

interactions between salts. Salt and osmotic stress limits the mobilization of reserves in several species (Lin and Kao 1995). Our results confirm these findings showing an inhibited breakdown of lipid in seeds in response to osmotic and salt (NaCl in particular) stress. These effects may be the consequence of the reduced enzymatic activities of glyoxylate cycle, which operates in the conversion of fats to carbohydrates during the germination of oil-rich seeds (Chia *et al*. 2005).

Treating seeds with chemical media in order to enhance their germination rate damaged the embryos. The 2, 3, 5–triphenyltetra-zolium chloride tests showed that the seed embryos were killed by chemical media used in the experiment (Chapter 2).

We used a spatially explicit simulation model to examine the relative importance of vegetative growth in *B. juncea* var. Ensabi. Based on an understanding of the life cycle of this variety; we simulated growth and the relative contribution of vegetative growth by seedlings across the range of full sunlight and partial sunlight. *B. juncea* var. Ensabi exhibited a vigorous vegetative growth pattern during 4-10 weeks of their life cycle in both full-sun-exposed plants (FEP) and partially-sun-exposed plants (PEP). Under the different ratios of light, there was a remarkable difference in leaf number of *B. juncea* var. Ensabi. The numbers of leaves were different at various phenological and morphological stages and the trends were nonlinear regression models. The total number of leaves in FEP was increasing faster than PEP. Under low light conditions, the life duration is shorter than that of full-sun-exposed plants.

The growth potential of *B. juncea* var. Ensabi was not without constraints. The generally sigmoid patterns of vegetative growth of the plant with time were manifestations of such constraints acting on and operating in the plant, perhaps as the consequence of intra-plant and inter-modular competition, life history function and time-mediated depletion of finite resources in the system (Chapter 2).

Allometric growth was prevailed in *B. juncea* var. Ensabi, invested proportionally more in above-ground parts in terms of number of pods, seeds, 2^{o} branch, leaves/2^{o} branch, leaves /1^{o} branch, pods/2^{o} branch and total leaves in each plant in FEP, whereas the PEP had a higher plant height than did the FEP.

Survival and growth depend on genetic potential of plants to adjust to environmental changes such as phytochrome that plays a very important role, but other factors such as light other than red and far-red, wind, temperature and 'transpiration demand' may also be important in stabilizing plant size inequalities in the field (Awada 2000).

The source of nitrogen (N) used in soil fertility practices affects plant growth, nutrient absorption, and the availability of nutrients. **(**Hamlin and Barker 2006). The suppressive effect of high N supply on plant growth was demonstrated clearly with *B. juncea*. Plant growth also was influenced by the accumulation of N, P, and K ions in plant tissues.

In this study, yield and yield component of *B. juncea* var. Ensabi were increased as the proportion of fertilizer increased in rates from 0 to 150 kg of the total NPK plus 150 kg N ha^{-1} supplied more than farmers experience used. Based on these results, N application higher than optimal dose had adverse effect on yield of *B. juncea* var. Ensabi.

The results of this experiment led to a general conclusion that for successful cultivation of *B. juncea* var. Ensabi, it should be fertilized 300 kg NPK (15-15-15) ha^{-1} plus 150 kg N ha^{-1} in splits *i.e.* full NPK with half N at sowing while the remaining half of N fertilizer was applied 25 days after planting, because N is commonly the most limiting plant nutrient, farmers generally apply fertilizers based on N requirements of a crop. Full dose of NPK should be applied in the row below the seed in the form of ammonium nitrate (34% N), triple super phosphate and muriate of potash as the source of N, P and K respectively. As sulphur is one of the essential nutrients for plant growth and brassica crops show a high demand for it. It is also essential for glucosinolate biosynthesis, and

the influence of sulphur fertilization on glucosinolate content has been widely reported. Based on reports of many researchers (Jackson 2000; Govahi and Safari 2006; Mahi *et al.* 2007), *B. juncea* has been shown respond markedly to sulphur fertilization. It is clear more research is needed to understand the importance of nutrition status of *B. juncea* var. Ensabi based on micro nutrient and sulphur.

Plant competition for light is a phenomenon occurring commonly in natural and agricultural vegetations. The effect of inter-plant spacing on the growth of individual plants in an increased space and equal sample size (number of plants) for each spacing gradient was investigated under varied plant spacing. Spacing gradients have been used to predict yield density relationships by excluding plants on and near the edges.

Increasing plant densities appeared to have a strong influence on both the vegetative of *B. juncea* var. Ensabi (Chapters 4, 5). Widely spaced plants were significantly taller than more closely spaced plants of the same age. Mean stem diameter, plant biomass, number of leaves and branches, and branch length increased with increase in plant (wider) spacing. The relationship between mean plant biomass and spacing followed a monotonic function, but in trend of the number of leaves showed fluctuations up and down the expected trend. This indicated that interference in this kind of design depends on the variable and may or may not follow a monotonic function of inter-plant distance. The species showed a very high survival percentage even when planted in high densities. As plant density increased, the rate of mortality increased as the result of self thinning that happened in two plants in arcs 1, 2. The occurrence of self-thinning was probably due to prevailing conditions such as low light quantum, competition for space and nutrients created by the neighbouring plants grown in the smaller available area.

It seems reasonable to assume that some kind of regulatory mechanism was operating in *B. juncea* var. Ensabi, which determined vegetative as a response to density; the mechanics of such

regulations were probably through an integrative hormone control or as local adjustments and responses to the presence of neighbours. The display of a sigmoid pattern of commutation in the numbers of leaves, biomass and branches, with time, for all densities were thought to be reflections of intra-plant competition operating in *B. juncea* var. Ensabi. As sigmoidal growth propels a population toward saturation density, the realizable ability of individuals in the population to reproduce or survive presumably declines.

Further, the inability of individuals of the species sown at low spacing (higher densities) to proliferate at rates similar to those grown at wide space or lower densities was thought to be a consequence of earlier inter-plant interference from neighbours in the higher-density regimes (Chapters 4 and 5). Harper (1977), Baki (1986) and Faravani (2008) have put similar arguments on density-dependent plant interference.

Under high densities, the stresses of intra-specific competition resulted in death of the less competitive members of the population. This density-dependent mortality has been termed "self-thinning". The -3/2 power law has generally been used to describe density-dependent mortality occurring as the result of intra-specific competition (Harper 1977). Mortality during the phase of self-thinning is largely among individuals suppressed by the ensuing growth of neighbours, resulting in increased shading within the canopies of neighbouring plants. The different self-thinning power is a plant response to the resource utilization and sensitivity to stress. A strongly negative density-dependent linear relationship with time was observed in the first and ensuing harvests at the five-density treatment over the period of 100 days after sowing (Chapter 4). This negative relation was possibly due to inhibition of the CO_2 produced by the roots, and competition for resources between seeds and seedlings might be implicated (Inouye 1980; Lonsdale and Watkinson 1982). The decreased allocation of resources to sexual reproduction is a common response to high levels of intra-specific competition (Harper 1977).

The relationship between plant biomass and plant density regimes was allometric with the slope value of -1.4 within the 95% confidence limits of the slopes of the significant models at $p<$ 0.05. It is envisaged that each population will start to thin along a line of slope from – 2.9 to -1.4 until it reaches the maximum standing crop (**Table 4.2**). It matches well with findings based on a much longer observation period on stands of shrub species in a different area (Marquet *et al.* 2005). The self-thinning slope is more widely accepted, on theoretical grounds, as being -1/2 (or -3/2), as originally suggested by Yoda *et al*. (1963). The relationship between mean shoot weight per plant and the density of survivors in the populations conforms to the power law. Self-thinning occurred along a line with a slope of -1.29 (R^2=0.63, $p<0.05$) and 95% confidence limits to slope -2.50 (or -0.09) an intercept, *log* k, of 8.83 and then deflect from it as dead genets accumulate within the population. The experiment was conducted inside an insect-proof house with reduced light intensities. Maximum biomass was reduced and the slope was not exactly -1, but it is close to -1 or -4/3. Recently, the traditional slope of -3/2 has been changed to -4/3, deduced from some new mechanical theories, such as the metabolic theory. It is difficult to obtain the accurate self-thinning exponent by fitting to data points directly, because of the intrinsic problem of subjectivity in data selection. More well controlled experiments should be carried out in order to identify the more precise slope of power law that might appear between -3/2 and -4/3 (Chen *et al.* 2008).

A slope of -3/2 indicates that in a growing, self-thinning population, mean plant weight increases faster than the decrease in density. A population following a -3/2 thinning line will therefore steadily increase its total weight. The thinning line might be expected to change from slope of -3/2 to a slope of -1, in such a way that the increase in mean plant weight is likely compensated by the decrease in density. A slope of -1 indicates that the further growth of survivors is exactly balanced by the deaths of other individuals. Upon reaching the asymptote with the maximum total

yield possible, no further increase is possible for the species in question in that environment (Begon *et al.* 1990).

These results support the concept of the competition-mediated self-thinning rule but more well controlled experiments should be carried out in order to identify which is the more accurate value between −3/2 and −4/3 or -1.

The oil concentrations of the *B. juncea* var. Ensabi seeds was 34.6% (Chapter 6). This is an extraordinarily high quantity of oil by a crop that is known as a vegetable. The protein concentrations of the Ensabi seeds was 32.1% (**Table 6.2, Chapter 6**) and we found that *B. juncea* var. Ensabi seed oils contain fatty acids commonly found in other seed oils.

Our assessment of the nutritional characteristics of the Ensabi oil will not fundamentally change, although the presence of linoleic acid, albeit in low concentrations, does increase the marketability of the oil from a nutraceutical standpoint and in contrast, presence of high amount of glucosinolate makes it nutritionally low quality oil.

The fatty acid profiles of *B. juncea* var. Ensabi seed oils are nutritionally outstanding, with a 22% concentration of polyunsaturated fatty acids (PUFAs) and very low concentrations of saturated fatty acids (SFAs). The saturated fatty acid concentrations were exceptionally low in this crop and well below the concentrations found in widely consumed vegetable oils, e.g., soybean and cottonseed oil. The saturated fat contents of the Ensabi seed oils rival those of high oleic sunflower oil and Canola oil.

The fatty acid profiles of *B. juncea* var. Ensabi seed oil showed the two human essential PUFAs, *i.e.*, C18:2 (ω-6) and C 18:3 (ω-3) were presented in a ratio of 3:1. As the linoleic and linolenic acid are essential fatty acids and are required in a ~3 to 1 ratio in the human diet, *B. juncea* var. Ensabi oils can be promoted as a source of perfect oil for human nutrition.

Many results of this research project merit further investigation. For example, the high contents of long-chain fatty acids, especially erucic acid, and glucosinolates that remain in the cake after oil extraction from brassica oilseeds are two factors that severely limit development of oilseed brassica as a competitive protein and oil break crop. Whereas the oil extracted from Ensabi consists of higher amounts of glucosinolate and erucic acid, it would be useful to explore mechanisms that led to control and reduce these compounds.

Allelopathy plays a significant role in the agroecosystems leading to a wide array of interactions among crops, weeds and trees. Generally, these interactions are deleterious to the receiver plants but may also provide a selective advantage to the donor. In agroecosystems, it leads to the problem of soil sickness or causes the autotoxicity that adversely affect the crops and thus their yield (Kohli *et al.* 2006).

A number of crops have been observed to exhibit allelopathic effect on other crops and weeds, besides being autotoxic. Of these interactions, crop autotoxicity in agroecosystems is significant (Batish *et al.* 2001). The principal causes of crop autotoxicity include the deliberate leaving of crop residues or old roots in soil releasing phytotoxins which may directly affect the succeeding crops, cause microbial imbalance, change organic matter of soil, increase ion leakage, disturb nutrient uptake and immobilization (Kohli *et al.* 2006).

Some allelochemicals present in *B. juncea* var. Ensabi extracts may directly prevent germination of other species when environmental conditions are conducive to growth and establishment (Chapter 6). It may have an important role indirectly, in determining plant community structures. Radish (*R. sativus*) seeds germination was inhibited at concentrations ranging 200 – 300 gL^{-1} (in the aqueous extracts of dried materials) to 18 – 30 gL^{-1} (in the ethanol extracts). The inhibition of root and shoot growth was also observed in barnyard grass seed. Both species were susceptible to allelopathy by extracts isolated from shoot and root of *B. juncea* var. Ensabi and their

rate of germination, root length and shoot length decreased with application of both types of extracts. The results from this study strongly suggest that allelopathy may be a possible mechanism controlling the timing of barnyardgrass (*E. crus-galli*) germination and seedling establishment.

Our findings of the allelopathic effect of *B. juncea* var. Ensabi are consistent with similar effect reported for other species (Chon *et al.* 2005).

Allelopathic compounds are secondary plant products released into environment through volatilization, leaching, root exudation and decomposition of plant residues in soil. These metabolites, such as phenolics, flavonoids, alkaloids, terpenoids and cyanogenic glycosides have often attracted scientists to elucidate their structure and biological function.

Several allelochemical compounds have been identified and separated from *B. juncea* extracts (Uppestrom 1997), and we found phenolic compounds in crude extracts of Ensabi. Possibly, the phenollic compounds inhibit root elongation and cell division completely, indicating that the thickness of seminal roots was enlarged abnormally. It is thought that only transverse growth of roots was sequentially maintained while longitudinal growth was greatly inhibited by these extracts and the phenolic content (Chon *et al.* 2002). The phenolic content might be the main factor influencing the growth and number of a weed species in the infested area. Plant phenolic compounds are among the major allelochemicals implicated in allelopathy (Inderjit and Mukerji 2006). Plant phenolic compounds have been widely reported to be substances inhibitory of seed germination and plant growth by affecting photosynthesis, protein synthesis, mineral uptake, chlorophyll content, membrane permeability and water utilization in plants (Tet-Vun and Ismail 2006).

In the present study, the emergence and growth of all the bioassayed species (radish and barnyardgrass) were inhibited by the ethanolic extracts of *B. juncea* var. Ensabi. It has been shown to have inhibitory effect on the germination and growth of other plant species, and this observation supported the previous studies which showed that phenolic compounds released by dominant plants

could inhibit germination, suppress the growth of other plants and finally eliminate the sensitive plants altogether (Reigosa *et al.* 1999).

Our findings on allelopathy of *B. juncea* var. Ensabi have often concentrated on the elucidation of allelopathic mechanism in seed germination and seedling growth. The future prospective researches can be emphasized as follows:

Further studies on isolation and identification of allelopathic compounds in *B. juncea* var. Ensabi need to be carried out. Although some biologically active compounds have been found, we still need to explore new compounds from this crop.

Investigating the naturally occurring allelopathic compounds of var. Ensabi that can use as agrochemicals which constituents a variety of natural products used as herbicides, insecticides, fungicides and nematicide.

The allelopathy may regulate the density and production of plant community under the canopy of a dominant species and limits the population of its associated species, but the allelopathic compounds often do not kill the seeds of plant species (Chou, 1999). Therefore, allelopathic compounds of Ensabi are better used as naturally biological control agent without extincting the affected species. In addition, allelopathy also acts as an evolutionary strategy for species survival under a dominant plant.

There are many important action mode for allelopathic compounds in plants (Reigosa *et al.* 2006), such as: (1) effect of allelopathic compounds on division, elongation and ultra structure of cells, (2) effect of growth inhibitors on hormon-induced growth, (3) effect of allelopathic compounds on membrane permeability, (4) effect of allelopathic compounds on mineral uptake, (5) effect on photosynthesis, (6) effect on respiration, (7) effect on protein synthesis, lipid and organic acid metabolism, (8) inhibition or stimulation of specific enzyme activity, (9) effect on water relationship,

(10) effect on DNA or RNA synthesis. However, the mode of action of phytochemicals present in *B. juncea* var. Ensabi is not well understood.

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APPENDICES

Appendix 1. The world's *Brassica* aggregates as vegetables*

Common name	Genus	Species	Variety
Kale	*Brassica*	*oleracea*	*acephala*
Collards	*Brassica*	*oleracea*	*acephala*
Chinese broccoli (gai laan)	*Brassica*	*oleracea*	*alboglabra*
Cabbage	*Brassica*	*oleracea*	*capitata*
Brussel sprout	*Brassica*	*oleracea*	*gemmifera*
Kohlrabi	*Brassica*	*oleracea*	*gongylodes*
Broccoli	*Brassica*	*oleracea*	*italica*
Broccoflower	*Brassica*	*oleracea*	*italica* x *botrytis*
Broccoli romanesco	*Brassica*	*oleracea*	*botrytis*/*italica*
Cauliflower	*Brassica*	*oleracea*	*Botrytis*
Wild broccoli	*Brassica*	*oleracea*	*oleracea*
Bok choy	*Brassica*	*rapa*	*chinensis*
Mizuna	*Brassica*	*rapa*	*nipposinica*
Broccoli rabe	*Brassica*	*rapa*	*parachinensis*

Appendix.1 (*cont.).*

Common name	Genus	Species	Variety
Flowering cabbage	*Brassica*	*rapa*	*parachinensis*
Chinese cabbage, napa cabbage	*Brassica*	*rapa*	*pekinensis*
Turnip root; greens	*Brassica*	*rapa*	*rapifera*
Rutabaga	*Brassica*	*napus*	*napobrassica*
Siberian kale	*Brassica*	*napus*	*pabularia*
Canola/rape seeds; greens	*Brassica*	*napus*	*oleifera*
Wrapped heart mustard cabbage	*Brassica*	*juncea*	*rugosa*
Mustard seeds, brown; greens	*Brassica*	*juncea*	
Mustard seeds, white	*Brassica*	*hirta*	
Mustard seeds, black	*Brassica*	*nigra*	
Tatsoi	*Brassica*	*rosularis*	
Ethiopian mustard	*Brassica*	*carinata*	

*Adapted and updated from Barlo (1999)

Appendix 2. *Brassica juncea* binomials and varieties*

Brassica juncea (L.) Czern	Big-Stem Mustard Group	Subsp. tatsai Mao
Brassica juncea (L.) Czern	Curly-Leaf Group	var. *crispifolia* L.H. Bailey
Brassica juncea (L.) Czern.	Cut-Leaf Mustard Group	var. *japonica* (Thunb.) L.H. Bailey
Brassica juncea (L.) Czern.	Head Mustard Group	var. rugosa (Roxb.) N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Head Mustard Group	var. *rugosa* (Roxb.) N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Head Mustard Group	var. *rugosa* (Roxb.) N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Large-Petiole Mustard Group	var. strumata N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Large-Petiole Mustard Group	var. strumata N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Large-Petiole Mustard Group	var. strumata N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Leaf Mustard Group	var. foliosa L.H. Bailey
Brassica juncea (L.) Czern.	Multi-Shoot Mustard Group	var. multiceps N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Mustard Group	var. juncea
Brassica juncea (L.) Czern.	Raya Group	var. juncea
Brassica juncea (L.) Czern.	Root Mustard Group	var. napiformis (Pailleux & Bois) Kitam.
Brassica juncea (L.) Czern.	Crispifolia Group	var. crispifolia L.H. Bailey
Brassica juncea (L.) Czern.	Foliosa Group	var. foliosa L.H. Bailey
Brassica juncea (L.) Czern.	Foliosa Group	var. foliosa L.H. Bailey
Brassica juncea (L.) Czern.	Juncea Group	var. juncea

Appendix 2. (*cont.*).		
Brassica juncea (L.) Czern.	Juncea Group	var. juncea
Brassica juncea (L.) Czern.	Rugosa Group	var. rugosa (Roxb.) N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Strumata Group	var. strumata N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Tumida Group	var. tumida N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.	Tumida Group	var. tumida N. Tsen & S. N. Lee
Brassica juncea (L.)Czern.		subsp. integrifolia (H. West) Thell
Brassica juncea (L.)Czern.	Chirimenna Group	subsp. integrifolia (H. West) Thell.
Brassica juncea (L.) Czern.	Crispifolia Group	subsp. integrifolia (H. West) Thell.
Brassica juncea (L.) Czern.	Crispifolia Group)	subsp. Integrifolia (H. West) Thell.
Brassica juncea (L.) Czern.	Fimbriata Group)	subsp. integrifolia (H. West) Thell.
Brassica juncea (L.) Czern.	Crispifolia Group)	subsp. integrifolia (H. West) Thell.
Brassica juncea (L.) Czern.		subsp. napiformis (Paill. & Bois) Gladis
Brassica juncea (L.) Czern. subsp. rugosa (Roxb.) Prain		var. rugosa (Roxb.) N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.		subsp. tatsai Mao
Brassica juncea (L.) Czern.		var. agrestis Prain
Brassica juncea (L.) Czern.		var. capitata N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.		var. chirimenna Makino
Brassica juncea (L.) Czern.		var. celerifolia Tsen & Lee
Brassica juncea (L.) Czern.		var. cernua Forb. et Hemsl.

Appendix 2. (*cont.).*		
Brassica juncea (L.) Czern.		var. cernua Forb. et Hemsl.
Brassica juncea (L.) Czern.		var. crispifolia L.H. Bailey
Brassica juncea (L.) Czern.		var. cuneifolia (Roxb.) Kitam.
Brassica juncea (L.) Czern.		var. foliosa L.H. Bailey
Brassica juncea (L.) Czern.		var. gracilis Tsen & Lee
Brassica juncea (L.) Czern		var. integrifolia (H. West) Sinskaya
Brassica juncea (L.) Czern.		var. japonica (Thunb.) L.H. Bailey
Brassica juncea (L.) Czern.		var. juncea
Brassica juncea (L.) Czern.		var. linearifolia Sun ->
Brassica juncea (L.) Czern.		var. longidens L.H. Baile
Brassica juncea (L.) Czern.	subsp. napiformis (Paill. & Bois) Gladis	var. megarrhiza N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.		var. multiceps N. Tsen & S. N. Lee
Brassica juncea (L.) Czern.		var. multisecta Bailey
Brassica juncea (L.) Czern.	subsp. napiformis (Paill. & Bois) Gladis	var. napiformis (Pailleux & Bois) Kitam.
Brassica juncea (L.) Czern.		var. oleifera Prain
Brassica juncea (L.) Czern.		var. rugosa (Roxb.) N. Tsen & S. N. Lee
Brassica juncea (L.) Czern		var. sareptana Sinskaja
Brassica juncea (L.) Czern		var. rugosa (Roxb.) N. Tsen & S. N. Lee
Brassica juncea (L.) Czern		var. strumata N. Tsen & S. N. Lee
Brassica juncea (L.) Czern	subsp. tatsai Mao	var. tsatsai Mao

*Adapted and updated from Barlo (1999)

Appendix 3. Botanical and vernacular names of *Brassica juncea* species and subspecies*

Species and sub-species	Language	Common/Vernacular name
Brassica juncea (L.) Czern.	Chinese	Jie cai, Gai cai, Tian jie cai
	Czech	Brukev sítinovitá
	English	Brown mustard, Indian mustard
	Finnish	Mustasinappi
	French	Moutarde brune, Moutarde jonciforme, Chou des Indes
	German	Brauner Senf, Indischer Senf
	Hebrew	Kruv samrani
	Hungarian	Indiai mustár
	Italian	Senape indiana, Senape bruna
	Japanese	Karashina, Seiyou karashina
	Khmer	Khat naa
	Laotian	Kaad khièw
	Malay	Sawi, Ensabi, Sawi pahit
	Nepalese	Asal raaii, Laahaa
	Polish	Kapusta sitowata
	Portuguese	Mostarda Indiana
	Russian	Gorchítsa
	Spanish	Mostaza de la China, Mostaza de la India, Mostaza hindu
	Tagalog	Mustasa
	Thai	Phakkat khieo, Phakkat khieopli
	Turkish	Yaprak hardal
Brassica juncea (L.) Czern. subsp. integrifolia Synonym(s) : *Brassica juncea* (L.) Czern. var. foliosa, *Brassica juncea* (L.) Czern. var. integrifolia, *Brassica juncea* (L.) Czern. var. rugosa	Bengali	Laaii
	Chinese	Da wang jie
	English	Leaf mustard
	Hindi	Baralaaii, Raaii
	Japanese	Setsuriko
Brassica juncea (L.) Czern. var. cuneifolia	English	Wedge-shape leaved mustard
Brassica juncea (L.) Czern. var. foliosa	Chinese	Da wang jie
	English	Plain-leaved mustard
	Japanese	Setsuriko

Appendix 3. (*cont.).*

Species and sub-species	Language	Common/Vernacular name
Brassica juncea (L.) Czern. subsp. Integrifolia Synonym(s): *Brassica juncea* (L.) Czern. var. crispifolia, *Brassica juncea* (L.) Czern. var. fimbriata , *Brassica juncea* (L.) Czern. var. chirimenna, *Brassica juncea* (L.) Czern. var. cernua	Chinese Dutch English French German Japanese	Yang jie cai, Juan bian ye jie-cai Krulmosterd. Curled mustard, Curly-leaved mustard Moutarde frisée, Moutarde - plume Krausblättriche-Senf Hagoromo karashina
Brassica juncea (L.) Czern. var. gracilis	Chinese English French	You jie cai, Gao you cai Slender mustard, Oil mustard Moutarde oléifère de Chine
Brassica juncea (L.) Czern. var. japonica Synonym(s): *Brassica japonica*, *Sinapis japonica*	Chinese French Japanese	Shui cai Moutarde des rizières Irana
Brassica juncea (L.) Czern. var. juncea Synonym(s) : *Brassica juncea* (L.) Czern. var. agrestis		Most names generally applied to the species Brassica juncea (L.) Czern. can be applied to this variety
Brassica juncea (L.) Czern. var. linearifolia	Chinese English	Sheng cai, Shui cai Narrow-leaved mustard, Japanese water cabbage
Brassica juncea (L.) Czern. var. longidens	Chinese English	Ke jia jie cai Hakka mustard
Brassica juncea (L.) Czern. var. megarrhiza	Chinese English French	Da tou cai, Jing yong jie cai Sichuan large-rooted mustard, Turnip-rooted mustard Moutarde tubéreuse de Chine

Appendix 3. (*cont.).*

Species and sub-species	Language	Common/Vernacular name
Brassica juncea (L.) Czern. var. multiceps	Chinese	Jiu tou jie, Xue li hong (Cantonese Suet lui hung, Hsueh li hung)
	English	Nine-headed mustard, Red in snow, Serrated-leaved mustard, Multishoot mustard
Brassica juncea (L.) Czern. var. multisecta	Chinese	Duo lie ye jie, Duo lie jie, Yin si jie (Cantonese Ngan sz kaai), Jin si jie, Qian jin cai (Cantonese Ts'in kan ts'oi), Xue li hong (Cantonese Suet lui hung, Hsueh li hung)
	English	Cut-leaved green in snow, Thousand nerved cabbage, Silverthread mustard
	French	Moutarde de Chine à mille têtes
Brassica juncea (L.) Czern. var. rugosa Synonym(s) : *Brassica juncea* (L.) Czern. subsp. rugosa, *Brassica juncea* (L.) Czern. var. capitata, *Brassica juncea* (L.) Czern. (Capitata Group), *Brassica rugosa*	Chinese	Bao xin jie cai, Chang jiao jie cai, Da xin jie cai, Da jie cai,Kuan ye jie cai, Chao zhou da jie cai (Cantonese Chiu chau taai kaai ts'oi)
	English	Cabbage leaf mustard, Heading leaf mustard, Broad-leaved mustard, Swatow mustard
	French	Moutarde chou, Moutarde à feuilles larges
	German	Breitblättrige-Senf
	Hindi	Pahaadii raaii

Appendix 3. (*cont.).*

Species and sub-species	Language	Common/Vernacular name
Brassica juncea (L.) Czern. subsp. Napiformis Synonym(s) : *Brassica juncea* (L.) Czern. var. megarrhiza *Brassica juncea* (L.) Czern. var. napiformis	Chinese English French	Ou zhou da tou cai, Yang da tou cai, Gen yong jie cai, Chong cai Pailleux's large-rooted mustard, Turnip-rooted mustard Moutarde tubéreuse
Brassica juncea (L.) Czern. et Cosson var. sareptana Sinskaja	Chinese Danish Dutch English Estonian Finnish French German Hungarian Italian Norwegian Polish Russian Spanish Swedish	Mei cai Sareptasennep Sarepta mosterd, Sareptamosterd Sarepta mustard, Lyrate-leaved mustard Sarepta kapsasrohi Sareptansinappi Moutarde sarepta Sareptasenf, Sarepta-Senf Szareptai mustár Senape Indiana Sareptasennep Gorczyca sarepska, Kapusta sarepska Gorchítsa sareptskaia Mostaza de Sarepta Sareptasenap
Brassica juncea (L.) Czern. var. strumata Synonim(s): *Brassica juncea* (L.) Czern. (Strumata Group)	Chinese English	Bao bao qing cai Horned mustard, Large-petioled mustard, Szechuan mustard

Appendix 3. (*cont.)*.

Species and sub-species	Language	Common/Vernacular name
Brassica juncea (L.) Czern. subsp. tatsai Mao	Chinese	Zha cai (Cha tsoi)
	English	Sichuan pickling mustard,
Synonym(s):		Sichuan swollen stem mustard,
		Big stem mustard, Yangtze
Brassica juncea (L.) Czern. var. tsatsai ined., *Brassica juncea* (L.) Czern. var. tsatsai Mao, *Brassica juncea* (L.) Czern. var. tumid	French	river mustard
		Moutarde à pied renflé

* Multilingual Multiscript Plant Name Database 2007.

Appendix 4.

Preparation of sample for gas chromatography:

One milliliter of 0.5 M KOH was added to 0.5 mL of sample and was incubated at 86^{o}C for 10 min. Upon cooling, 2 mL of hexane was added, vortexed for 15 min and centrifuged to remove the hexane phase. To aqueous layer, 1 mL of 0.7 M NH_4C1 and 2 mL of hexane were added followed by centrifugation at 5000 rpm for 15 min. The hexane layer was collected and left evaporated. Then 0.2 mL of benzene and 0.5 mL of BF_3 were added and incubated at 86°C for 10 min followed by addition of 1 mL of water upon cooling. The aqueous phase was extracted 3 times with 2 mL of hexane. The hexane layer was pooled and 5 mL of water was added. This layer was then separated after centrifugation. Benzene was added to the evaporated hexane layer to enable sample application for gas chromatography (Goud *et al*. 2007).

Appendix 5.

Kjeldahl method

The Kjeldahl method was developed in 1883 by Johan Kjeldahl. This method used for the determination of nitrogen, is applicable to many types of organic compounds, although the method is sometimes referred to for the determination of aminoid nitrogen.

Apparatus:

Digestion flasks. Kjeldahl hard, moderately thick, well annealed glass flasks with a total capacity of ca 500.

Distillation flasks. Same as Kjeldahl flask fitted with rubber stopper through which passes lower end of efficient connecting bulb or trap to prevent mechanical carry over of NaOH during distillation. Connected upper end of bulb to condenser tube with rubber tubing. Used graduated 500 mL Erlenmeyer titration flask to collect distillate. Trapped outlet of condenser in a manner to ensure complete absorption of NH_3 distilled into boric acid solution.

Digestion/distillation system. Traditional apparatus with adjustable controls for individual flasks.

Reagents:

Sulfuric acid, Concentrated: Reagent grade (96% H_2SO_4)

Potassium sulfate: Reagent grade (K_2SO_4), free from nitrogen

Copper selenite: Reagent grade ($CuSeO_3$ $2H_2O$)

Sodium hydroxide solution, 50% (w/v)

Sodium hydroxide solution, 0.1 N: Standard

Sulfuric acid solution, 0.1 N: Standard

Methyl red-bromocresol green indicator: Dissolved 0.33 g bromocresol green and 0.66 g methyl red dyes in 1 liter of 95% ethyl alcohol. Added sufficient 0.1 N sodium hydroxide solution to produce green colour followed by the dropwise addition of 0.1 N hydrochloric acid solution to produce a deep wine-red colour.

Zinc metal: Granular, 20 mesh.

Procedure: Grinded about 50.0 g of sample through a laboratory-cutting mill and mixed thoroughly. Determined moisture content of grounded sample by an approved method. Weighed about 2.0 g of sample and transfered to the digestion flask. Added 10.0 g of potassium sulfate and 0.3 g of copper selenite followed by the addition of 30 mL of concentrated sulfuric acid. Placed flask in inclined position on digestion unit and heated below boiling until frothing had ceased. Increased heat until acid boiled briskly and digested for 90 minutes after the reaction mixture cleared. Measured accurately 25.0 mL of standard 0.1 N sulfuric acid solution into a 500-mL Erlenmeyer flask. Connected flask to distillation assembly so that the condenser delivery tube was immersed in the absorbing acid.

Cooled the digest in the Kjeldahl flask, diluted carefully with about 300 mL of purified water, mixed thoroughly and added a pinch of granular zinc to prevent bumping during distillation. Added sufficient volume of 50% sodium hydroxide solution to make the mixture strongly alkaline, pouring it down the side of the flask to avoid immediate mixing with the acid solution. Connected flask to condenser by means of connecting bulb, turned on heater and mixed contents of the flask gently by swirling. Distilled at a moderate rate until all ammonia had passed into the absorbing solution. About 250 mL of distillate was collected.

Removed receiving flask and titrated excess acid with standard 0.1 N sodium hydroxide solution using about 0.25 mL of methyl red-bromocresol green mixed indicator. Conducted a blank

determination on all reagents, substituting pure sucrose or dextrose for the sample and calculated the 0.1 N sulfuric acid equivalence (blank).

Calculation:

% Nitrogen (dry basis) = (mL of 0.1 H_2 SO_4 - Blank - mL of 0.1 NaOH) × 0.0014 ×100 ±×100/ Sample Wt. (g) × (100 - Sample Moisture, %)

% Protein = % Nitrogen × 6.25

Appendix 6

Glucosinolate measurement

Reagents:

a. Acidified 40% (v/v) methanol (mixed 400 mL methanol with 500 mL water, cooled and added 5.0 mL of glacial acetic acid and diluted to 1.0 liter with water). Approximate pH 3.0.
b. Test combination glucose; GOD-perid. Obtained from Boehringer, Germany.
c. Sinigrin (Allay glucosinolate potassium salt monohydrate. M.W. 416).
d. Myrosinase

Procedure:

a. Following is the protocol of the complete procedure.

Procedure	Time (min)	Sample	Sample blank
Weigh	2	1.0 g seed	1.0 g seed
Myrosinase hydrolysis	7	Added 1.0 mL of water	Added 1.0 mL of acidified methanol
Filtration and dilution	5	Added 19.0 mL of acidified methanol, mixed, filtered and diluted filtrate 10 fold	
Colorimeter procedure & measurement	16	Took 0.2 mL diluted filter for GOD-perid glucose determination	
Total time	30 min		

Extraction and hydrolysis:

Transferred two accurately weighed 1.0 g samples of whole seeds into separate loaded ball-mill cups. To one cup (a) added 1.0 mL of water (sample), and to the another cup (b) added 1.0 mL of acidified 40% (v/v) methanol/water (sample blank). Ball-mill side by side for 2 min allowed to stand 5 min, then to each cup, added 19.0 mL of acidified 40% (v/v) methanol. Recapped, hold firmly and shaken vigorously to mix. Filtered the cup contents through charcoal-coated papers,

suitably folded to give rapid filtration and discarded the first few mL of filtrate. These acidified methanolic extracts were stable for many hours after filtration enabling several samples to be assayed by the colorimetric glucose procedure simultaneously.

Colorimetric glucose assay:

Prior to colorimetric assay, each of the filtrate was diluted ten-fold with water and then pipetted 0.2 mL into separate 10-mL tubes. 0.2 mL water was pipetted into a third tube (water blank) and 0.2 mL standard glucose solution, taken directly from the Test combination, into a fourth tube. Added 5.0 mL of buffer/enzyme/chromogen reagent prepared from the Test combination to all tubes. Covered with stopper, mixed and incubated at 37^{o}C water-bath and read within 10-15 min. Alternatively, tubes were left at room temperature and read within 25-30 min.

Measured the absorbance, A of each solution against the water blank at 610 nm in a 1-cm cell.

Calculation:

Glucose (µg) produced by myrosinase hydrolysis, allowing for free glucose, in the 0.2 mL aliquot of diluted extract taken for colorimetric assay:

$$\frac{A(sample) - A(sample\ blank)}{A(standard)} \times 18.2$$

µmol total glucosinolate g^{-1}seed = $\frac{\mu g\ glucose\ produced}{1\ (sample\ wt\ g)} \times \frac{20}{1} \times \frac{10}{0.2} \times \frac{1}{180\ (M.W.glucose)}$

or

$$\frac{A(sample) - A(sample\ blank)}{A(standard)} \times 101$$

ccc